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Drinking Water Criteria Document for Copper (Final Draft)

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Cincinnati, OH

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DRINKING WATER CRITERIA DOCUMENT FOR COPPER

Prepared for

OFFICE OF DRINKING WATER

Prepared by

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Assessment Office
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The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on copper.
This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of copper
- Toxicokinetics and human exposure to copper
- Health Effects of copper in humans and animals
- Mechanisms of toxicity of copper
- Quantification of toxicological effects of copper

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TABLE OF CONTENTS

Page

I. SUMMARY I-1

II. PHYSICAL AND CHEMICAL PROPERTIES. II-1

STRUCTURE AND IDENTIFICATION II-1
PHYSICAL AND CHEMICAL PROPERTIES II-1
STABILITY IN WATER II-1
SUMMARY. II-12

III. TOXICOKINETICS. III-1

ABSORPTION III-1
DISTRIBUTION III-5
BIOACCUMULATION/RETENTION. III-7
METABOLISM III-8
EXCRETION. III-10
SUMMARY. III-14

IV. HUMAN EXPOSURE. IV-1

EXPOSURE ESTIMATION IV-1

Drinking Water IV-1
Diet IV-6
Air. IV-8

SUMMARY IV-8
REFERENCES. IV-12

V. HEALTH EFFECTS IN ANIMALS V-1

GENERAL TOXICITY V-1

Acute Toxicity. V-1
Chronic and Subchronic Toxicity V-10

TARGET ORGAN TOXICITY. V-11

Liver V-11
Kidney. V-12
Blood V-12
Lungs V-13
Gastrointestinal Tract. V-14
Central Nervous System. V-14

Page

OTHER EFFECTS. V-15

Carcinogenicity V-15
Mutagenicity. V-16
Teratogenicity and Reproductive Toxicity. V-21

SUMMARY. V-26

VI. HEALTH EFFECTS IN HUMANS. VI-1

CLINICAL CASE STUDIES. VI-1

Ingestion VI-1
Inhalation. VI-9
Dermal Absorption VI-11

EPIDEMIOLOGICAL STUDIES. VI-12
HIGH RISK SUBPOPULATIONS VI-12

Wilson's Disease Patients VI-12
Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency. VI-14
Kidney Dialysis Patients. VI-14
Occupational Exposure VI-15

SUMMARY. VI-15

VII. MECHANISMS OF TOXICITY. VII-1

HEPATOTOXICITY VII-2

Animal Model for Hepatotoxicity VII-2

BLOOD TOXICITY VII-2

Glucose 6-Phosphate Dehydrogenase Deficiency (G6PD) VII-3
Animal Models VII-3

WILSON'S DISEASE VII-4

Animal Models VII-4

MENKES SYNDROME. VII-5

Animal Model. VII-5

SUMMARY. VII-5

Page

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS VIII-1

NONCARCINOGENIC EFFECTS. VIII-4
QUANTIFICATION OF NONCARCINOGENIC EFFECTS. VIII-8

Calculation of the 1-day HA VIII-8
Calculation of the 10-day HA. VIII-10
Calculation of Lifetime AADI. VIII-11

CARCINOGENIC EFFECTS VIII-14
QUANTIFICATION OF CARCINOGENIC EFFECT. VIII-14
SPECIAL CONSIDERATIONS VIII-14

Synergistic Effects VIII-14
Special Groups At Risk. VIII-15

SUMMARY. VIII-15

IX. REFERENCES. IX-1

LIST OF TABLES

No.	Title	Page
II-1	Copper Compounds.	II-2
II-2	Copper Compounds: Physical and Chemical Properties	II-8
III-1	Copper in Various Human Tissues	III-9
IV-1	Copper Levels in Adult Male, Infant and Toddler Diets by Fiscal Year.	IV-9
IV-2	Estimated Intake of Copper from the Environment by Adult Males in μg/kg/day.	IV-10
V-1	Toxicity Data in Rats	V-3
V-2	Acute and Subchronic Oral Toxicity Data in Pigs	V-8
V-3	Tumorigenicity of Some Copper Compounds	V-17
V-4	Mutagenicity Data for Copper Compounds.	V-18
V-5	Teratogenicity Data for Copper Compounds.	V-21
VI-1	Human Dose Effect Data for Copper	VI-4

LIST OF ABBREVIATIONS

AADI	Adjusted acceptable daily intake
ADI	Acceptable daily intake
bw	Body weight
CNS	Central nervous system
DNA	Deoxyribonucleic acid
FEL	Frank-effect level
HA	Health advisory
i.p.	Intraperitoneal
IUD	Intrauterine device
i.v.	Intravenous
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
ppm	Parts per million
RNA	Ribonucleic acid

I. SUMMARY

In environmental waters, copper is present almost exclusively as the Cu^{2+} ion. The ion is generally a complex of organic or inorganic ligands but sometimes is present as the aqua ion $[Cu(H_2O)_6]^{2+}$. Complexes of the carbonate appear to be most common, although many other complexes are present, particularly in organic rich water. Hydrous manganese and iron oxides appear to have dominating control of soluble copper, strongly sorbing the complexed ion; however, in organically rich sediments copper sorbed to the oxides may become redissolved. Clay and particulate organic material also sorb copper.

Municipal water treatment plants that use iron chloride coagulation may concentrate copper present in the raw water onto the coagulate, based on the sorption properties of copper to hydrous iron oxides. The majority of copper present in drinking water appears to come from copper water pipes.

Copper is regarded as an essential element for proper nutrition, and it is widely distributed in the body. Copper compounds can be absorbed by all three environmental routes; dermal, oral and inhalation. Although not thoroughly understood, homeostatic mechanisms in normal humans provide a balance between copper intake and elimination. Normal daily intakes of copper are 2-5 mg/day, mostly from food and water, with a slight contribution from air. Normal humans store ~70-120 mg of copper, most of which is located in the liver.

Acute toxicity from ingestion of excess copper can result in gastrointestinal disturbance, hemolysis, glucose-6-phosphate dehydrogenase inhibition, kidney and renal necrosis, and death. Chronic ingestion of copper compounds rarely results in toxic effects, and then only in those individuals with genetic intolerance (Wilson's Disease). The chronic occupational use of copper aerosols may result in adverse effects on the lungs, kidney and liver of exposed persons.

Copper plays an important role in reproduction. Deficiency or excess of the metal can have a teratogenic effect. The spermicidal properties of copper have long been recognized.

Pertinent data regarding the carcinogenicity of ingested copper compounds could not be located in the available literature. The equivocal mutagenic responses of microbial, mammalian cell cultures and in vivo systems do not permit a conclusion regarding the mutagenicity of copper.

The available human data indicate that gastrointestinal distress occurred following ingestion of solutions that contained 5-7 mg of copper and other unknown chemicals as a single dose. These data were used to derive a 1-day health advisory (HA) of 1.3 mg/ℓ which, when spread over a day, should provide protection from these acute effects. Since the effects appear to be local irritation, this HA is recommended for both the child and the adult. There are no data indicating that human exposure to copper results in chronic toxic effects. Furthermore, there is a nutritional requirement for copper and acute effects appear to be the effects of concern. Therefore, the 1-day HA, 1.3 mg/ℓ, will also be used as the AADI.

II. PHYSICAL AND CHEMICAL PROPERTIES

Structure and Identification

Copper and some of its important compounds are listed in Table 2-1, with, where available, their Chemical Abstracts Service (CAS) numbers.

Physical and Chemical Properties

The chemical and physical properties of copper and some if its important organic and inorganic compounds are listed in Table 2-2.

Stability in Water

The dominant removal processes for copper in water include the following: sorption and coprecipitation by hydrous iron and manganese oxides, adsorption to clays, minerals and organic solids and ion exchange in the crystal lattice of carbonates. Adsorption occurs primarily when copper is complexed, but uncomplexed copper will also adsorb. Copper is not expected to volatilize or photolyze (Callahan et al., 1979).

In environmental waters, copper in solution is present as the Cu^{2+} aqua ion $[Cu(H_2O)_6]^{2+}$; the lifetime of the Cu^{2+} aqua ion depends on the conditions, but it will last for a few hours, at most, if air is excluded (Cotton and Wilkinson, 1980). Other copper valance states are possible (Cu^{3+} and Cu^{4+}) but rare. Consequently, whenever copper is mentioned in this discussion, the Cu^{2+} ion is meant unless otherwise specified.

Many organic and inorganic copper complexes can be formed by treating aqueous copper solutions with ligands (Cotton and Wilkinson, 1980). Stiff

TABLE II-1

Copper Compounds*

Compound	CAS No.	RTECS No.
Copper	7440-50-8	GL5325000
Copper abietate	10248-55-2	
Copper(I) acetate	598-54-9	
Copper(II) acetate	142-71-2	
Copper(II) acetate, basic	52503-63-6	
Copper(II) acetate monohydrate	6046-93-1	
Copper acetate oxide	6533-47-7	
Copper(II) acetoarsenite	12002-03-8 1299-88-3	 GL6475000
Copper(II) acetylacetonate	13395-16-9	
Copper(I) acetylide	1117-94-8	
Copper(II) amine sulfate	14283-05-7	
Copper amino acid chelate	-	
Copper(I) ammonium acetate	43043-77-2	
Copper(II) ammonium acetate	23087-46-9	
Copper ammonium arsenate	32680-29-8	
Copper ammonium carbonate	33113-08-5	
Copper(I) ammonium chloride	-	
Copper(II) ammonium chloride	-	
Copper ammonium hydroxide	17500-49-1	
Copper(II) ammonium sulfate	-	
Copper(I) antimonide	12054-25-0	
Copper(II) antimonide	12054-21-6	
Copper(II) arsenate	10103-61-4 7778-41-8	
Copper arsenate hydroxide	16102-91-4	
Copper(I) arsenic sulfide	-	
Copper(I) arsenide	12005-75-3	
Copper(II) arsenide	12254-86-3	
Copper(II) arsenite	10290-12-7 1302-97-2	 CG3385000
Copper aspinnate	23642-01-5	
Copper(II) benzoate	533-01-7	
Copper(II) benzoyl acetonate	14128-84-8	
Copper bicarbonate	7492-68-4	
Copper bis(salicylaldehyde)	14523-25-2	
Copper(II) borate	39290-85-2	
Copper bromate	-	
Copper(I) bromide	7787-70-4	
Copper(II) bromide	7789-45-9	
Copper(I) tert-butoxide	-	
Copper butylphthalate	-	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper butyrate	540-16-9	
Copper iso-butyrate	-	
Copper caprylate	-	
Copper(II) carbonate	1184-64-1	
	16211-10-2	
Copper(II) carbonate, basic	12069-69-1	
Copper chelate	-	
Copper(II) chlorate	14721-21-2	
Copper(II) chlorate, tetrahydrate	10294-45-8	
Copper(I) chloride	7758-89-6	
Copper(II) chloride	7447-39-4	
	12258-96-7	
Copper(II) chloride, basic	16004-08-3	
	1332-65-6	GL7020000
	12167-76-9	
	12356-86-4	
Copper(II) chromate(III)	12018-10-9	
Copper(II) chromate(VI)	13548-42-0	
	12617-87-7	
	13675-47-3	
Copper(II) chromate, basic	1308-09-4	
Copper chromite	11104-65-7	
Copper(II) chromite	12018-10-9	
Copper chromite barium	-	
Copper chromite zinc oxide	-	
Copper(II) citrate	866-82-0	GL7056000
Copper(II) citrate, hydrate	6020-30-0	
Copper cobalticyanide	-	
Copper(II) columbate	-	
Copper(I) cyanide	544-92-3	
Copper(II) cyanide	4367-08-2	GL7175000
Copper(II) cyclohexylbutyrate	2218-80-6	
Copper(II) dichromate	13675-47-3	
Copper(II) dichromate, dihydrate	12018-10-9	
Copper dihydrazinium sulfate	-	
Copper diiron tetraoxide	-	
Copper(II) dimethyldithiocarbamate	137-29-1	FA0175000
Copper disodium ethylenediamine tetracetate	4025-15-1	
Copper(II) ethylacetoacetate	-	
Copper(II) ethylacetyl acetonate	14284-35-3	
Copper(II) ethylenediamine	14552-35-3	
Copper ethylenediamine-N,N'-diacetic acid	32575-57-8	
Copper ethylenediamine gold cyanide	18974-18-0	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper ethylediamine nickel cyanide	63427-32-7	
Copper ethylenediamine silver cyanide	67859-40-9	
Copper ethylenediamine sulfate cyanide	-	
Copper ethylenediamine tetracetate	54452-03-1	
Copper ethylenediamine zinc cyanide	67859-43-2	
Copper(II) 2-ethylhexanoate	149-11-1 22221-10-9	
Copper ethyl xanthate	-	
Copper(II) ferrate (III)	12018-79-0	
Copper(II) ferricyanide	-	
Copper ferrite	-	
Copper(II) ferrocyanate	-	
Copper(II) ferrocyanide	13601-13-3	
Copper(II) ferrous sulfide	1308-56-1	
Copper(I) fluoborate	14708-11-3	
Copper(II) fluoborate	38465-60-0	
Copper(II) fluoride	7789-19-7	
Copper(II) fluosilicate	12062-24-7	
Copper(II) formate	544-19-4	
Copper(II) formate tetrahydrate	5893-61-8	
Copper gallium selenide	12018-83-6	
Copper gallium sulfide	12158-59-7	
Copper gallium telluride	12018-84-7	
Copper(II) gluconate	527-09-3	
Copper(II) gluconate monohydrate	6020-31-1	
Copper glutanate	-	
Copper(II) glycinate	13479-54-4	
Copper glycolate	-	
Copper guaiacolsulfonate	-	
Copper heptanoate	5128-10-9	
Copper(II) hexacyanoferrate(II)	13601-13-3	
Copper hexafluoroacetylacetone		
Copper(I) hydride	13517-00-5	
Copper hydroselenite	10031-38-6	
Copper hydroselenite monohydrate	10031-39-7	
Copper hydroselenite dihydrate	10031-40-0	
Copper hydroselenite trihydrate	10031-41-1	
Copper(II) hydroxide	20427-59-2 1344-69-0	
Copper(II) hydroxyfluoride	13867-72-6	
Copper(II) 8-hydroxyquinolate	10380-28-6	
Copper(II) hydroxysalicylate	62320-94-9	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper indium selenide	-	
Copper indium sulfide	-	
Copper indium telluride	-	
Copper(II) iodate	7681-65-4	
Copper(I) iodide	13767-71-0	
Copper(II) iron(III) oxide	-	
Copper(II) lactate	-	
Copper(II) linoleate	7721-15-5	
Copper malonate	7268-92-0	
Copper(II) magnesium sulfate	-	
Copper mercaptobenzothiazolate	4162-43-0	
Coper(I) mercuric iodide	13876-85-2	
Copper methane arsenate	-	
Copper(II) methylate	-	
Copper(II) molybdate	13767-34-5	
Copper(II) naphthenate	1338-02-9	
Copper(II) neodecanoate	32276-75-8 50315-14-5 68084-48-0	
Copper(II) nickel formate	68134-59-8	
Copper(II) nitrate	3251-23-8	
Copper(II) nitrate trihydrate	10031-43-3	
Copper(II) nitrate hexahydrate	13478-38-1	
Copper(I) nitride	1308-80-1	
Copper(II) nitride	-	
Copper(II) nitroprusside	14709-56-9	
Copper cis-9-octadecenoate	1120-44-1	
Copper octanoate	20543-04-8	
Copper(II) oleate	1120-44-1 10402-16-1	
Copper(II) oxalate	814-91-5	
Copper(I) oxide	1317-39-1 1317-38-0	
Copper(II) oxychloridesulfate	8012-69-9	
Copper(II) palmitate	-	
Copper(II) pentamethylene dithiocarbamate	-	
Copper peponate	-	
Copper(II) perchlorate	13770-18-8	
Copper(II) perchlorate hexahydrate	10294-46-9	
Copper(II) permanganate	-	
Copper(II) phenolsulfonate	547-56-8	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper(I) phenylacetylide	13146-23-1	
Copper 3-phenylsalicylate	5328-04-1	
Copper phorphine	13007-96-0	
Copper phosphate	7798-23-4 18718-12-2	
Copper(II) phosphate, ortho	10103-48-7	
Copper(II) phosphate, pyro	10102-90-6	
Copper(I) phosphide	12019-57-7	
Copper(II) phosphide	12134-35-9	
Copper phthalate	-	
Copper(II) phthalocyanine	147-14-8	
Copper(II) potassium chloride	-	
Copper(I) potassium cyanide	13682-73-0 14263-73-1	
Copper potassium hexacyanoferrate(II)	14481-39-1	
Copper potassium pyrophosphate	-	
Copper(II) potassium sulfate	-	
Copper potassium tartrate	-	
Copper powder	7440-50-8	
Copper(II) propionate	3112-74-1	
Copper iso-propoxide	-	
Copper proteinate	-	
Copper(II) resinate	9007-39-0	
Copper b-resorcylate	-	
Copper ricinoleate	-	
Copper rubidium sulfate	-	
Copper(II) salicylate	16048-96-7	
Copper(II) selenate	15123-69-0	
Copper(II) selenate pentahydrate	10031-45-5	
Copper(I) selenide	20405-64-5	
Copper(II) selenide	1317-41-5	
Copper(II) selenite	10214-40-1	
Copper(II) silicate	1344-72-5 16509-17-4	
Copper silicide	-	
Copper(II) sodium chloride	-	
Copper(I) sodium cyanide	-	
Copper sodium tartrate	-	
Copper(II) stannate	12109-07-7	
Copper(I) stearate	20563-00-2	
Copper(II) stearate	660-60-6	
Copper(II) sulfamate	-	
Copper(II) sulfate	7758-98-7	
Copper(II) pentahydrate	7758-99-8	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper sulfate, basic	1332-14-5 1344-73-6	GL8930000
Copper(I) sulfate b-naphthol	-	
Copper(I) sulfide	22205-45-4	
Copper(II) sulfide	1317-40-4	
Copper(I) sulfite	13982-53-1	
Copper(I) sulfite, monohydrate	10294-49-2	
Copper tallate	61789-22-8	
Copper(II) tartrate	815-82-7	
Copper(I) telluride	12019-52-2	
Copper(II) telluride	12109-23-7	
Copper(II) tellurite	-	
Copper tetramine sulfate	14283-05-7	
Copper tetramine sulfate monohydrate	10380-29-7	
Copper meso-tetraphenylporphrine	-	
Copper(I) thiocyanate	1111-67-7	
Copper(II) thiocyanate	-	
Copper thiourea gold cyanide	68975-64-4	
Copper thiophenoxide	-	
Copper(II) titanate	12305-89-4	
Copper p-toluenesulfonate	-	
Copper triethanolamine	-	
Copper trifluoroacetylacetonate	-	
Copper 3,5,5-trimethylhexanoate	35206-70-3	
Copper(II) tungstate	13587-35-4	
Copper undecanoate	7491-40-9	
Copper undecylenate	1322-15-2 1328-71-8	
Copper(II) vanadate	12789-09-2	
Copper m-vanadate	-	
Copper zinc chromate	1336-14-7	
Copper zinc chromate(VI)	10279-64-8	
Copper zinc oxide	-	
Copper(II) zirconate	-	

*Source: U.S. EPA TSCA list and U.S. EPA (1980); SRI International (1980); USITC, 1980; Merck Index, 1976; Chem. Sources - USA, 1980

TABLE II-2

Copper Compounds: Physical and Chemical Properties

Compound	Description	Boiling Point (°C)	Melting Point (°C)	Solubility (g/100 g)	Specific Gravity	Molecular Weight
Copper	reddish, lustrous, ductile, malleable metal	2595	1083	insoluble in water; soluble in nitric acid, slightly soluble in HCl and NH_4OH	8.92	63.54
Copper(I) acetate	white to transparent crystals	NA	decomposes	decomposes	NA	122.6
Copper(II) acetate, basic	green to blue solid depending upon formula	NA	NA	slightly soluble	NA	369.26
Copper(II) acetoarsenite	emerald green powder	NA	NA	insoluble	NA	1013.71
Copper(II) acetylacetonate	crystalline powder	sublimes	>230	slightly soluble	NA	261.76
Copper(I) acetylide	red powder	NA	decomposes	very soluble	NA	151.10
Copper(I)ammonium acetate	NA	NA	NA	NA	NA	NA
Copper ammonium carbonate	NA	NA	NA	NA	NA	NA
Copper(II) arsenite	light green powder	NA	decomposes	insoluble	NA	187.46
Copper(II) bromide	black, deliquescent crystalline powder or crystals	900	498	very soluble	4.77	223.31
Copper(I) bromide	white powder or cubic crystals	1345	504	slightly soluble	4.72 to 4.98	143.46
Copper chlorides, basic	green-blue or yellow powder	NA	NA	insoluble	NA	variable
Copper(II) chromate(III)	grayish-black to blue-black crystals	NA	decomposes	insoluble	NA	231.56
Copper(II) chromate(VI)	reddish-brown crystals	NA	decomposes	insoluble	NA	179.55
Copper(II) chromate(VI), basic	light chocolate-brown powder	NA	decomposes	insoluble	NA	374.64
Copper(II) citrate	green or bluish-green crystals or powder	NA	decomposes	slight	NA	315.18
Copper(II) cyanide	green powder	NA	decomposes	insoluble	NA	115.58

TABLE II-2 (cont.)

Compound	Description	Boiling Point (°C)	Melting Point (°C)	Solubility (g/100 g)	Specific Gravity	Molecular Weight
Copper(II) dimethyldithiocarbamate	dark brown powder	NA	>325	insoluble	5.25	303.98
Copper disodium ethylenediamine tetracetate	NA	NA	NA	NA	NA	397.71
Copper ethylene diamine tetracetate	NA	NA	NA	NA	NA	353.75
Copper(II) heptanoate	NA	NA	NA	NA	NA	323.90
Copper(II) ferrocyanide	reddish-brown powder	NA	NA	insoluble	NA	339.04 (anhydride)
Copper(II) fluoborate	NA	NA	NA	NA	NA	NA
Copper(II) fluoride	anhydride - white crystals	NA	950 decomposes	4.7	4.23	101.54
	dihydride - blue crystals	NA	decomposes	4.7	2.93	137.57
Copper(II) formate	blue crystals	NA	NA	12.5	1.831	153.55 (anhydride)
Copper(II) gluconate	light blue to bluish-green powder	NA	NA	30 (at 25°C)	NA	453.58
Copper(II) glycinate	white (anhydride) to blue (hydrates) crystals	NA	NA	soluble	NA	211.66 (anhydride)
Copper(I) iodide	pure white to brownish-yellow powder	1290	606	0.0008 (at 18°C)	5.62	190.46
Copper(II) linoleate	NA	NA	NA	NA	NA	NA
Copper(II) metaborate	bluish-green crystalline powder	NA	NA	slightly insoluble	3.859	149.16
Copper(II) neodecanoate	solid	NA	NA	NA	NA	267.79
Copper(II) orthophosphate	light blue powder	NA	decomposes	insoluble	NA	434.61
Copper(II) oxalate	blue-white powder	NA	decomposes	0.00253 (at 25°C)	NA	160.57

TABLE II-2 (cont.)

Compound	Description	Boiling Point (°C)	Melting Point (°C)	Solubility (g/100 g)	Specific Gravity	Molecular Weight
Copper(II) phosphate	solid	NA	NA	NA	NA	267.79
Copper(II) phthalocyanine	blue-red crystalline	NA	NA	NA	NA	576.08
Copper(II) potassium cyanide	solid	NA	NA	NA	NA	154.68 or 284.92
Copper(II) pyrophosphate	NA	NA	NA	NA	NA	391.1
Copper(II) resinate	green powder	NA	NA	insoluble	NA	NA
Copper(II) stearate	light blue, amorphous powder	NA	125-250	insoluble	NA	630.50
Copper(II) sulfate, basic	fine, light-blue, gelatinous powder	NA	decomposes	insoluble	3.78	470.30
Copper(I) sulfide	bluish to grayish-black lustrous powder	NA	1100	insoluble	5.6	159.15
Copper(II) thallate	NA	NA	NA	NA	NA	NA
Copper(I) thiocyanate	yellow-white, amorphous powder	NA	1084	0.0005 (at 18°C)	2.85	121.62
Copper(II) 3,5,5-trimethylhexanoate	solid	NA	NA	NA	NA	380.01

NA = Information not readily available

(1971a,b) reported that copper in solution at environmental temperatures, pH and inorganic carbon content is present as complexes of copper carbonate rather than the aqua ion $[Cu(H_2O)_6]^{2+}$. In polluted waters or those with high organic content, copper is present as complexes with cyanide, amino acids and humic substances in addition to the carbonate complexes and the aqua ion. Stiff (1971b) also found that most of the copper present in rivers in England was associated with suspended solids and that dissolved copper was present as organic complexes. Stumm and Morgan (1981) report that the activity ratio diagram reveals that Cu^{2+} predominates up to pH=6; $CuCO_3$(aq) predominates in the pH range 6-9.3, and $Cu(CO_3)_2^{-2}$ (aq) in the pH range 9.3-10.7.

Sylva (1976) and Ramamoorthy and Kushner (1975) confirmed the importance of organic complexing in the speciation of copper in organic rich waters. In waters with low organic content, precipitation of malachite ($Cu_2(OH)_2CO_3$) appears to be dominant, although the rate appears to be very slow at low copper concentrations (Sylva, 1976).

Lopez and Lee (1977) found that copper speciated in the order $CuOH^+ > Cu^{2+} > CuCO_3$ in a copper polluted organic depleted lake. Soluble copper concentrations were controlled by sorption onto hydrous iron and manganese oxides.

The dominating effect of hydrous manganese and iron oxides in controlling soluble copper was also reported by Hem and Skougstad (1960), Jenne (1968), Brown et al. (1983), Lee (1975), Carpenter et al. (1975), Steele and Wagner (1975) and Collins (1973). These oxides appear to be most important in water with low organic content and soils. In reducing or acidic environments (i.e., organically rich sediments), copper sorbed to hydrous iron and manganese oxides may become redissolved, allowing the copper to reenter the water.

Brown et al. (1983), Baes and Sharp (1983), Payne and Pickering (1975) and Huang et al. (1977) reported that copper was strongly adsorbed to clay materials and that this sorption was strongly pH dependent. The addition of certain particulate organic materials increased the sorption. Soluble organic matter, however, appears to increase efficiently the solubility of copper, possibly by competing for clay active sites (Jackson and Skippen, 1978).

Sewage treatment plant sludge has long been known to concentrate many heavy metals including copper (Bradford et al., 1975; Sopper, 1973; Page, 1974; Lester et al., 1979). In addition, based on the above discussion, municipal drinking water plants that practice iron chloride coagulation may concentrate copper present in the raw water on the coagulate, thus reducing the dissolved copper present in the finished water. Nonetheless, water, particularly water that is acidic, low in hardness and alkalinity, and consequently corrosive to piping, may leach copper from drinking water pipes and supply copper levels in >1.7 mg/ℓ (Karalekas et al., 1983). Sharrett et al. (1982) found that copper levels in drinking water were an order of magnitude lower when transported through galvanized pipe as opposed to copper pipe. Standing water gave levels about twice as high as did flowing water in copper pipe, but had little effect in galvanized pipe. The largest factor affecting copper concentrations in drinking water was found to be piping (Karalekas et al., 1983; Sharrett et al., 1982).

Summary

In environmental waters, copper is present, almost exclusively, as the Cu^{2+} ion (Cotton and Wilkinson, 1980). The ion is generally a complex of organic or inorganic ligands but sometimes is present as the aqua ion $[Cu(H_2O)_6]^{2+}$. Complexes of the carbonate appear to be most common (Stiff, 1971a,b), although many other complexes are present, particularly in organic rich water (Stiff, 1971a; Sylva, 1976; Ramamoorthy and Kushner, 1975). Hydrous manganese and iron oxides appear to have dominating control of soluble copper, strongly sorbing the complex ion (Hem and Skougstad, 1960; Jenne, 1968; Brown et al., 1983; Lee, 1975); however, in organically rich sediments copper sorbed to the oxides may become redissolved. Clay and particulate organic material also sorb copper (Brown et al., 1983; Sharp, 1983; Payne and Pickering, 1975).

Municipal water treatment plants that use iron chloride coagulation may concentrate copper present in the raw water onto the coagulate, based on the sorption properties of copper to hydrous iron oxides. The majority of copper present in drinking water appears to originate from copper water pipes (Karalekas et al., 1983; Sharrett et al., 1982).

III. TOXICOKINETICS

Absorption

Copper may be absorbed into the systemic circulation from oral administration, inhalation, or contact, and possibly from intrauterine contraceptive devices (i.e., IUDs).

Bearn and Kunkel (1954) characterized the uptake of ^{64}Cu by serum in rats and human subjects given an oral dose of (^{64}Cu) copper nitrate. Rats and human subjects were given 25.6 (2 m Ci) and 12.8 (1 m Ci) mg of ^{64}Cu, respectively. Blood samples were taken at intervals up to 24 hours and were assayed for radioactivity. Blood serum was subjected to electrophoretic separation and the fractions obtained were assayed for radioactivity. In both rats and men, the serum ^{64}Cu was localized in the albumin fraction 15 minutes after administration. After this period, serum ^{64}Cu began to localize in the ceruloplasmin fraction in both rats and man. By 24 hours after administration, at least 70% of the serum ^{64}Cu was localized in the ceruloplasmin fraction.

Weber et al. (1969) studied the gastrointestinal absorption of radiocopper in human subjects given oral doses of ^{64}Cu as copper acetate. Seven patients who had been fasting and who had experienced no liver damage or related problems were given (^{95}Zr) zirconium oxalate (0.5-10 µCi, specific activity not reported) followed by (^{64}Cu) copper acetate (100-500 µCi, specific activity 0.3-1.3 µ Ci/µg Cu). Zirconium oxalate was used as a nonabsorbed stool marker to compensate for the short half-life of ^{64}Cu (12.8 hours). The radionuclides were counted each day for 4 days in a whole body scintillation counter using gamma ray spectroscopy. In addition, the movement of orally administered ^{64}Cu in the gastrointestinal tract was monitored with scintiphotographs obtained with a scintillation camera. Urine and stools were collected each day, blood samples were taken hourly for 6 hours, and the ^{64}Cu content in plasma, urine and stools was counted by gamma ray spectroscopy. One week after the oral dosing, the subjects were again given similar oral doses of (^{95}Zr) zirconium oxalate and an intravenous dose of 500 μCi of (^{64}Cu) copper acetate that had been incubated with 10-20 mℓ of the subject's plasma for 30 minutes (to assure protein binding of copper). Radioactivity was monitored as before.

When ^{64}Cu was administered orally, maximal absorption of the metal from the stomach and duodenum by plasma was observed within 1 hour of administration. A second phase of absorption, which occurred at a lower rate, was observed for $\geq$3.5 hours after administration. At 2 hours after administration, the bolus of ^{64}Cu seen in scintiphotographs was present in the small intestine, and after 5 hours, in the ileocecal region and first portion of the large intestine. After i.v. administration, an average of 11.5% of the dose was excreted into the gastrointestinal tract (range 0-23%). The average net absorption of ^{64}Cu was ~60% (range 15-97%). Obviously, a wide variation in copper absorption was observed in these normal subjects. A steady state ^{64}Cu plasma concentration was observed after ~3 hours of oral or i.v. administration. The initial decline of ^{64}Cu plasma concentration after i.v. administration occurred rapidly and was observed to follow first order kinetics. Negligible urinary excretion of ^{64}Cu was observed.

In mammals, the sites of copper absorption following oral administration are limited to the upper gastrointestinal tract (Evans, 1973). The absorption of copper from the gastrointestinal tract is a complex process controlled by a number of mechanisms (Crampton et al., 1965). Copper absorption is facilitated by simple diffusion of ionic copper and by active transport of ionic and organic copper complexes with amino acids and proteins; it may be controlled by an intestinal protein called metallothionein. Metallothionein may control copper homeostasis by providing binding sites in intestinal mucosa for ionic copper, and by acting as a mucosal block to prevent absorption of toxic amounts of copper when present in excess quantities (Evans, 1973). Competition for intestinal binding sites by other metals (cadmium, zinc, mercury, silver) can alter the amount of copper absorbed. The amount of dietary protein can affect copper absorption as well, by forming protein complexes with the metal and thus inhibiting its absorption. Other factors that may affect copper absorption include the number and type of anions present (i.e., inhibition by sulfite ions), and ascorbic acid (Evans, 1973). It is believed that these mammalian homeostatic mechanisms control the copper balance in normal individuals despite large variations in dietary intake (Sternlieb, 1980). Studies with radiolabeled ^{64}Cu and ^{67}Cu showed that in adults ~60% of human dietary copper is not absorbed and is eliminated by the feces (Scheinberg and Sternlieb, 1969).

Batsura (1969) produced evidence of the pulmonary uptake of copper oxide in rats exposed to aerosols containing 50-80 mg/m^3. Animals were exposed for 15, 30, 45 or 60 minutes and killed immediately; another group was exposed for 180 minutes and killed at 0, 3, 6, 12, 18 or 24 hours after exposure. Electron microscopic histologic examination showed that absorption of copper had occurred in animals exposed for 180 minutes. Copper oxide particles penetrated the epithelial cells of alveoli and were found in plasma 6 hours after exposure began. Copper oxide was also observed in the proximal convoluted tubules of the kidney.

Data regarding the absorption of inhaled copper are scarce. Villar (1974) reported observing copper-containing granulomas in the lung, liver and kidney of a deceased patient who had been occupationally exposed to Bordeaux mixture (an aqueous solution of lime and 1-2% copper sulfate) during the spraying of vineyards. Pimental and Menezes (1975) reported finding copper-containing liver granulomas in three patients with a history of using Bordeaux mixture while involved in vineyard spraying. Gleason (1968) reported symptoms of metal fume fever (general discomfort, fever, sensations of chills or warmth and stuffiness of the head) in three workers exposed to fine copper dust at concentrations of 0.03-0.12 mg/m^3. Metal fume fever symptoms were absent in these workers after the installation of local exhaust ventilation, which reduced the airborne copper concentration to <0.008 mg/m^3.

A radiolabeled saline solution of copper complex (bis[glycinato]copper [II]) topically applied to the skin of cats was observed in the blood ~6 hours after administration. By 24 hours after administration, it was shown that ~3.3% of the applied copper had completely penetrated the skin (Walker et al., 1977). Copper has been shown to permeate intact (Walker et al., 1977) and burned (Holtzman et al., 1966) human skin. Apparently, copper bracelets used for relief of arthritic complaints are slowly dissolved and copper is subsequently absorbed (Walker et al., 1977).

Highly localized deposits of hepatic and renal copper have been observed in monkeys with copper IUDs and in control monkeys. Both copper and inert material IUDs have been observed to increase plasma copper levels (Oster and Salgo, 1977). This may be explained by the observation that stress or inflammation alone can result in increased serum copper levels (Evans, 1973).

Oreke et al. (1972) studied the systemic absorption of copper from radiolabeled (^{64}Cu or ^{67}Cu) copper wire that had been implanted into the uteri of Holtzman rats. Radiolabeled copper was found in the serum, liver and kidney of rats as early as 18.5 hours after implantation.

When included in plastic IUDs, copper results in more efficient contraception than plastic devices alone. The interaction of dissolved ions with blastocysts may prevent their implantation, and copper has spermicidal potential as well. Concern has been raised that systemic absorption of copper may occur in those women using IUDs (Oreke et al., 1972). Copper IUDs do have systemic effects but appear to be unrelated to the systemic accumulation of the metal (Oster and Salgo, 1977).

Distribution

Copper is normally present in all human tissues. In humans, the liver, brain, heart, kidney and adrenal contain the highest concentrations of copper. Tissues with moderate copper concentrations include intestine, lung and spleen. Low copper concentrations are found in endocrine glands, bone, muscle, larynx, trachea, aorta and testes (Schroeder et al., 1966; Evans, 1973). About 50% of the body burden of copper is found in muscle and bone because of the large masses involved. The liver contains ~10% of the body's copper stores (Evans, 1973).

Absorbed dietary copper is transported in the blood as complexes with plasma albumin (93%) and amino acids. These complexes include the immediate transport form of plasma copper, and albumin copper is in rapid equilibrium with tissue copper (Neumann and Sass-Kostsak, 1967; Evans, 1973). Copper is also contained in erythrocytes in the form of erythrocuprein (Shields et al., 1961) and other proteins (i.e., superoxide dismutase). Removal of copper from the serum takes place primarily in the liver, where it is either excreted into the bile, stored or used in synthesizing ceruloplasmin, which is released into the blood. Ceruloplasmin contains ~79% of the copper contained in the blood of a normal person.

Osborn et al. (1963) studied the hepatic uptake of radiolabeled copper in normal and Wilson's disease patients. Two normal subjects were given i.v. injections of 80-120 μCi of (^{64}Cu) copper chloride dissolved in saline. The livers of normal subjects had taken up 82-94% and 95-96% of the administered radioactivity within 24 and 30 hours of dosing, respectively. Patients with liver disease, heterozygous for Wilson's disease and other medical conditions, had reduced hepatic uptake of copper compared with normal subjects. Other observations that illustrate the puzzling aspects of Wilson's disease include chronic hepatic storage of copper with reduced serum ceruloplasmin levels.

When radiolabeled copper is administered to humans either orally or by i.v. injection, a rapid rise and fall of serum radioactivity in a 4-hour period is followed by a second phase wherin serum radioactivity rises more slowly (Bearn and Kunkel, 1954; Adelstein and Vallee, 1961). This second phase reflects the appearance of newly synthesized ceruloplasmin, which is released from the liver. Meanwhile, the concentration of liver radioactivity rises continuously, and small increases in bone marrow and erythrocyte radioactivity also occur (Bush et al., 1955; 1956; Adelstein and Vallee, 1961).

Bioaccumulation/Retention

Normal mammalian homeostatic mechanisms are effective in maintaining proper copper balance despite a wide range of dietary intake. A normal adult man (70 kg) is believed to store 70-120 mg of copper (U.S. EPA, 1980a or b). The liver functions as a depot for copper that synthesizes ceruloplasmin, the most abundant copper protein in the blood (U.S. EPA, 1980a or b).

Hepatic copper is distributed in several subcellular fractions associated with copper-dependent enzymes and copper-bound proteins (Evans, 1973). The large granule fraction (containing lysosomes and mitochondria) and nuclear fractions of normal mammalian livers each contain ~20% of the total hepatic copper. About 10% of hepatic copper is contained in the microsomal fraction. The cytosol fraction from normal mammalian liver contains the major portion of the total hepatic copper (Evans, 1973).

Factors that influence the amount of copper distributed in various body tissues include age, sex, amount of dietary copper and overall health of the individual. Neonates contain high concentrations of liver copper, ~6-10 times the amount found in a normal adult man (U.S. EPA, 1980a or b). These levels quickly decline within the first year of life, and a normal copper balance is then observed. The copper concentrations normally found in various human tissues are presented in Table III-1.

Metabolism

Copper is essential to the proper functioning of many important enzyme systems. It is well established that copper is required in the formation of hemoglobin, in pigment formation, in carbohydrate metabolism, in tissue respiration (Van Ravensteyn, 1944) and in catecholamine biosynthesis (Ahmed et al., 1981). Copper forms stable complexes and chelates with organic molecules such as amino acids, purines, pyrimidines, nucleotides, RNA, DNA and proteins (Evans, 1973). Several human copper proteins have been isolated including ceruloplasmin, superoxide dismutase, cytochrome oxidase, tyrosinase, monoamine oxidase and metallothionein (NAS, 1977).

It is difficult to prepare a diet adequate in all other respects that contains <2 mg of copper/day (Scheinberg and Sternlieb, 1969). However, some special diets or unusual dietary conditions may result in dietary deficiencies of copper (Williams, 1982). It is generally accepted that ingestion of 2.5-5 mg Cu/day will result in proper human copper balance. Many investigators have reported estimates of total body copper, and although subject to variation, a value of ~80 mg Cu in a 70 kg man is an apparent modal value (Williams, 1982).

The most important organ involved in copper metabolism is the liver. It functions as a storage depot, a site for ceruloplasmin synthesis (for copper

TABLE III-1

Copper in Various Human Tissues (ppm ash)*

	TISSUE						
	Liver	Brain	Heart	Kidney	Pancreas	Lung	Spleen
U.S. Children	1300	290	340	250	160	140	100
U.S. Adults	680	370	350	270	150	130	93

*Source: Shroeder et al., 1966

mobilization) and a site for forming a complex of copper with substances of variable molecular weight for subsequent biliary excretion (Dowdy, 1969).

Several investigators have attempted to characterize the copper compounds that leave the liver by the bile. The particular components include low molecular weight proteins and polypeptides, macromolecular complexes and bile acids and pigments (Sternlieb, 1980).

In vitro experiments in isolated perfused rat livers indicate that the copper put into storage, incorporated into ceruloplasmin or released in bile may originate in separate intracellular pools (Hazelrig et al., 1966). A three-compartment model has been postulated to explain this phenomenon. Under normal conditions, the three liver compartments and body tissues are in equilibrium. When excess copper is absorbed, the storage compartment accumulates the metal and eventually moves it to the other compartments for biliary excretion and ceruloplasmin synthesis. Ceruloplasmin is released into serum and is distributed. The liver may catabolize ceruloplasmin that is returned to the liver (Dowdy, 1966).

Mechanistic and quantitative models for human copper balance are presented in Figures III-1 and III-2.

Excretion

Copper is excreted from the body in bile, feces, sweat, hair, menses and urine. Biliary excretion is a major pathway for excretion of copper in man (Gollan and Deller, 1973). Estimates of the amount of biliary copper excretion range from 0.5-1.2 mg/day (U.S. EPA, 1980a or b). Human bile was

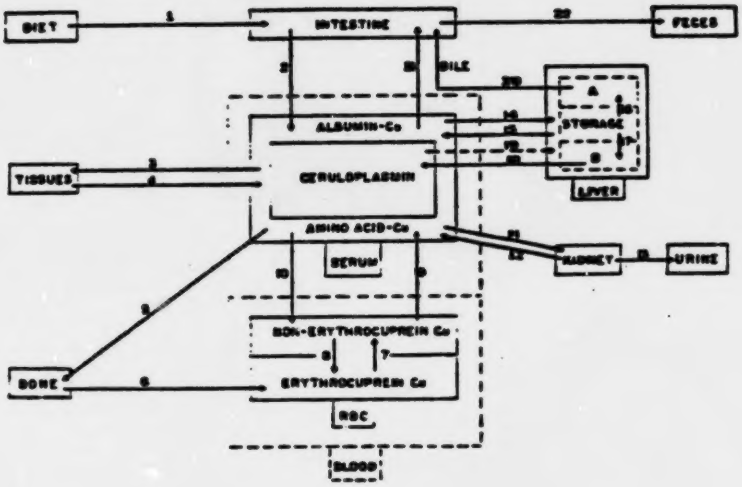
DIET
1
INTESTINE
22
FECES
2
BILE
A
STORAGE
B
LIVER
ALBUMIN-Cu
TISSUES
3
4
CERULOPLASMIN
AMINO ACID-Cu
SERUM
KIDNEY
URINE
10
9
5
NON-ERYTHROCUPREIN Cu
8
7
BONE
6
ERYTHROCUPREIN Cu
RBC
BLOOD

FIGURE III-1

Mechanisms of Human Copper Balance

Source: Dowdy, 1969

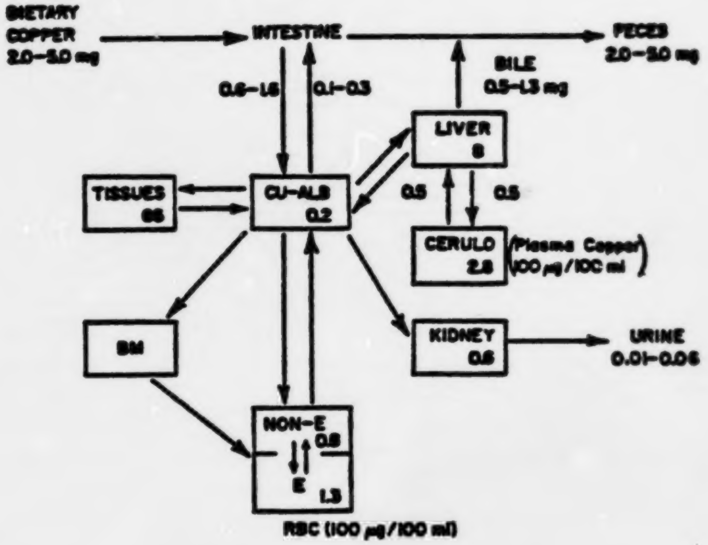
DIETARY COPPER 2.0-5.0 mg
INTESTINE
FECES 2.0-5.0 mg
0.6-1.6
0.1-0.3
BILE 0.5-1.3 mg
LIVER 8
TISSUES 66
CU-ALB 0.2
0.5
0.5
CERULO 2.8
(Plasma Copper 100 μg/100 ml)
BM
KIDNEY 0.6
URINE 0.01-0.06
NON-E 0.8
E 1.3
RBC (100 μg/100 ml)

FIGURE III-2

Schematic Representation of Some Metabolic Pathways of Copper.

The numbers in the boxes refer to milligrams of copper in the pool. The numbers next to the arrows refer to milligrams of copper transversing the pathway each day. CU-ALB, direct-acting fraction; CERULO, ceruloplasmin; NON-E, nonerythro-cuprein; E, erythrocuprein; BM, bone marrow; RBC, red blood cell.

Source: Herbert, 1970

labeled in vivo by administering 500 μCi of ^{64}Cu-cupric acetate (specific activity = 2.5 m Ci/mg) by i.v. injection to seven patients. The bile was collected at scheduled cholecystectomy operations, dialyzed and subjected to gel filtration with subsequent counting of radioactivity. About 2-4% of the administered radioactivity was present in gall bladder bile after 4 hours. Most (62-99%) of the ^{64}Cu in bile was in non-diffusible form, as observed in dialysis experiments. Gel filtration revealed the presence of two heat stable radioactive protein fractions, with molecular weights between 20,000 and 200,000. A smaller amount of radioactivity was excreted in a readily diffusible compound with low molecular weight.

Biliary copper travels to the intestine where it can be eliminated in the feces. Enterohepatic cycling does not occur to a great extent, particularly if adequate protein is present in the diet (Evans, 1973).

In adults, fecal excretion of unabsorbed and biliary copper probably represents ~60% of that taken in, as estimated by the difference between oral intake and fecal excretion (U.S. EPA, 1980b). Some excreted copper from saliva and gastric and intestinal mucosa may be present in the feces, but may only account for ~0.4 and 1 mg/day, respectively. Menstrual fluid may contain 0.1 mg Cu/month (U.S. EPA, 1980a or b).

Of the daily turnover of copper in normal persons, only ~4% is excreted by the urine (Dowdy, 1969). What little copper does appear in the urine may originate from amino acid-bound metal or from the dissociation of copper-albumin complexes. Little of the copper that is bound to ceruloplasmin or contained in erythrocytes will permeate the glomerulus, thus preventing excretion (Evans, 1973). Schroeder et al. (1966) estimated that daily copper excretion in normal humans from feces, urine and sweat is ~3640, 60 and 2 μg, respectively.

Summary

Administration of radiocopper to human subjects has been helpful in elucidating the mechanisms of copper disposition. Absorption of ingested copper occurs primarily in the upper portion of the gastrointestinal tract, with rapid appearance of copper bound to albumin and amino acids in the blood 1-2 hours after administration. Absorbed copper is transported by the blood to the liver, where it accumulates and results in decreased levels of blood copper. Blood copper levels then begin to rise slowly, reflecting the hepatic production and release of copper-bound ceruloplasmin. Copper is also incorporated into several other proteins, including cytochrome oxidase, monoamine oxidase, tyrosinase and erythrocyte superoxide dismutase. Biliary excretion of low molecular weight copper complexes occurs rapidly after administration, with increasing amounts of high molecular weight copper complexes being formed over time. Copper elimination occurs primarily by the fecal route. Negligible enterohepatic recycling of protein bound copper occurs in well-fed individuals. Relatively little copper is excreted by urine, sweat or menstrual fluid.

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as copper from a variety of sources, including drinking water, food, ambient air, occupational settings and consumer products. This analysis of human exposure to copper is limited to drinking water, food and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works and travels, what one eats, and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

In the Exposure Estimation Section of this chapter, available information is presented on the range of human exposure and intake for copper from drinking water, food and ambient air for the 70 kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combined exposures from those three sources. However, the Summary Section of this chapter provides some insight into the relative contributions of the three sources, especially drinking water, to the range of intake values suggested by the available data.

Exposure Estimation

Drinking Water. The U.S. EPA has established a National Secondary Drinking Water Standard of 1 mg/ℓ for copper (40 CFR 143). However, limited information was obtained on the occurrence of copper in U.S. drinking water supplies. One Federal survey [the 1969 Community Water Supply Survey (CWSS)] included copper as an analyte. Several additional national studies and state studies were reported in Ciccone and Associates (1984) and are presented below. According to Ciccone and Associates (1984), copper concentrations are not usually found in excess of 1.0 mg/ℓ by the time copper reaches a treatment plant.

The CWSS (McCabe et al., 1970) examined 120 surface water supplies, 613 groundwater supplies, 152 supplies with mixed water or purchased sources, and 84 special systems (trailer parks, tourist and other institutions) in the United States in 1969. Samples were analyzed for a number of contaminants, including copper. A total of 2595 distribution water samples were collected. Detailed results for all supplies sampled were not provided. However, McCabe et al. (1970) reported that 11 (or 1%) of the systems had levels in excess of 1.0 mg/ℓ, the U.S. Public Health Service's recommended limit for drinking water in effect at the time of this study. The number of individual samples exceeding the limit was 42 (or 1.6%), with a maximum value reported at 8.35 mg/ℓ.

In a nationwide study of interstate carrier water supply systems (U.S. EPA, 1975), 615 finished water sampling sites (water source unknown) were sampled in the 10 U.S. EPA regions. The total number of samples was not given and only the range of copper concentrations in the samples (0-0.78 mg/ℓ) was reported. The high value (0.78 mg/ℓ) was from a public utility in Alaska. The U.S. EPA secondary drinking water standard, or Maximum Contaminant Level (MCL), of 1.0 mg/ℓ was not exceeded in any sample.

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In another nationwide survey of trace elements in water, Kopp (1969) tested 380 finished water samples from surface water sources. Copper was detected in 65.2% of the samples with a mean concentration for positive samples of 0.043 mg/ℓ, and a range of 0.001-1.056 mg/ℓ.

In a study of public water supplies of the 100 largest cities in the United States (according to the 1960 U.S. census), Durfor and Becker (1962) sampled municipal water treatment facilities with various water sources. Copper was detected in 100% of the samples with a median value of 0.0083 mg/ℓ, and a range of <0.00061-0.25 mg/ℓ.

Barnett et al. (1969) characterized a Denver, Colorado public water supply during a 5-month period. Five finished water samples and four distribution samples were collected during the study (approximately monthly). Mean concentrations of copper reported were 0.0067 mg/ℓ (range of 0.002-0.02 mg/ℓ) for the surface water samples and 0.0081 mg/ℓ (range of 0.003-0.022 mg/ℓ) for the tap water samples. The authors noted that major portions of copper are removed during the treatment process when concentrations exceed 10 μg/ℓ (Ciccone and Associates, 1984).

Several corrosion case studies were also reported in Ciccone and Associates (1984). In a nationwide study of corrosion in water distribution systems, Patterson (1981) examined water samples from households served by municipal water supplies in 580 cities in 47 states. The study took place between May and November 1978. Sampling was performed in five regions of the United States: Northeast, Southeast, Midwest, South Central and West.

The number of samples taken from each region were in accordance with population size. Household systems were flushed for 30 seconds before sampling, and no samples were taken from homes using water softeners. For the total of 772 finished samples, the overall mean concentration of copper was 0.221 mg/ℓ; the concentrations ranged from not detected to 52.0 mg/ℓ. The lowest regional mean concentration (0.082 mg/ℓ) was reported in the South Central region for 84 samples; the values in this region ranged from not detected to 0.73 mg/ℓ. The highest regional mean concentration (0.526 mg/ℓ) was reported in the Northeast for 141 samples. The values in this region ranged from not detected to 52.0 mg/ℓ. Background metal concentrations were compensated for in the final analysis of corrosion products.

In a Seattle, Washington drinking water study of trace metal exposure, Sharrett et al. (1982) analyzed standing and running distribution water samples from a surface water source to compare the effects of galvanized piping and copper piping. Standing samples from each type of pipe were taken after no overnight use. Samples of running water followed 30-60 seconds of system flush. Three samples were taken at each of 74 distribution sites with galvanized pipes and 69 distribution sites with copper pipes. Mean values for the standing water samples from the galvanized and copper pipes were 0.224 and 2.192 mg/ℓ, respectively. Mean values for the running water samples from the galvanized and copper pipes were 0.172 and 1.343 mg/ℓ, respectively.

Cameron and Wunderlich (1976) associated copper concentrations as high as 3.5 mg/ℓ with copper piping corrosion. They also noted that samples from homes <5 years old were consistently higher in copper concentrations than samples from older homes.

Standing and running distribution water samples were examined from the Tolt and Cedar Rivers in Seattle, Washington, which serve as the total water supply for Seattle (Courchene et al., 1978). Standing samples were taken after 5-8 hours of stagnation time. Running samples were taken after the system was flushed for 5 minutes. Replicate samples were taken 5-7 times over a 1-year period at 145 distribution sites containing water from the Tolt River and at 134 distribution sites containing water from the Cedar River. Mean copper concentrations for the standing water samples from the Tolt and Cedar Rivers were both reported at 0.39 mg/ℓ, with ranges of 0.01-3.5 mg/ℓ and 0.01-1.9 mg/ℓ, respectively. Mean values for the running water samples were 0.12 mg/ℓ from the Tolt River and <0.045 mg/ℓ from the Cedar River, with ranges of 0-2.27 mg/ℓ and 0-0.29 mg/ℓ, respectively.

In another Tolt River study of corrosion products in the distribution system, Dangel (1975) compared standing and running distribution water samples from 32 sites with surface water (Tolt River) sources between October and December 1972. The supply studied provided approximately one-third of the total water supply to Seattle. Standing samples were taken after overnight contact with plumbing. Running samples were taken after 30 seconds of flushing. Two samples were collected at each site. The average levels of copper in the standing samples were more than three times higher than those in running samples, with reported mean concentrations of 0.45 mg/ℓ (range of 0.028-2.05 mg/ℓ) and 0.12 mg/ℓ (range of 0.003-1.67 mg/ℓ), respectively. Twenty-three percent of the standing samples exceeded 1.0 mg/ℓ.

The levels of copper observed in drinking water are generally in the low mg/ℓ range and below. However, copper levels may be substantially higher in distribution samples at certain locations, as indicated in corrosion case studies (e.g., 52 mg/ℓ). Assuming that copper is present at the highest concentration reported, i.e., 52 mg/ℓ in the Patterson corrosion study, the daily intake for the 70 kg adult male consuming 2 ℓ/day of drinking water would be 104 mg/day or 1.5 mg/kg/day.

Diet. Copper is commonly found in human foods. The concentration of copper in foods reflects the copper content of the soil or water in which they originated (Underwood, 1971). Crustaceans and shellfish (especially oysters), organ meats (especially lamb, beef or swine liver), nuts, dried legumes, dried vine and stone fruits, chocolate, mushrooms and unrefined cereals are particularly rich in copper (U.S. EPA, 1980; NAS, 1977, 1980). The copper content of these items can range from 20 mg/kg to as high as 400 mg/kg (McCance and Widdowson, 1947; Schroeder et al., 1966). Dairy products, white sugar and honey usually have extremely low levels of copper (NAS, 1980). The U.S. EPA (1980) reported that these foods rarely contain more than 0.5 mg/kg of copper. Nonleafy vegetables and most fresh fruits and refined cereals generally contain up to 2 mg/kg. Cheese (except Emmental), milk, beef, mutton, white and brown bread, and many breakfast cereals (unless they are fortified) are relatively poor sources of copper, i.e., they have <50 μg of copper/100 kcal (WHO, 1973).

Studies conducted between 1930 and 1970 on the dietary intake of copper generally concluded that the typical U.S. diet provided an intake of at least 2 mg/day of copper, the level that is considered to be adequate for normal copper metabolism (Adelstein et al., 1956). Tompsett (1934) reported that the typical daily intake of copper from food appeared to be 2-2.5 mg/day. The reported average intake of copper by young children, according to Daniels and Wright (1934) was 1.48 mg/day.

However, more recent studies indicate that U.S. diets may contain less than the required amount of copper. Klevay (1975) quotes studies showing that the dietary copper intake of high-school girls and college women may be as low as 0.34-0.58 mg/day, and that the diets of other individuals may supply <1 mg/day. Additional food analyses by Klevay have caused him to warn that U.S. diets may not adequately provide 2 mg/day of copper (Klevay, 1977; Klevay et al., 1977).

The Food and Drug Administration determined the levels of 11 essential minerals (including copper) in food commodity groups and total diets of adult males, infants and toddlers and reported this information in the Selected Minerals in Foods Survey, Fiscal Years 1974 through mid-1982. Pennington et al. (1984) summarized these data to evaluate the sources and adequacy of those minerals in the diets. Pennington and coworkers concluded that the three diets provided inadequate amounts of copper as compared to the 1980 estimated safe and adequate daily dietary intake (ESADDI) ranges. The ESADDI range for the adult diet was 2.0-3.0 mg/day; the ESADDI ranges for the infant and toddler diets were 0.7-1.0 mg/day and 1.0-1.5 mg/day, respectively (Food and Nutrition Board, 1980). The levels of copper in the three diets were below the lower end of the respective ESADDI ranges. The toddler diet contained 65-76% of the lower end copper concentration for toddlers, while the infant and adult diets contained 77-80% and 76-90%, respectively, of their lower end copper concentrations.

Table IV-1 summarizes the average levels of copper in the adult male diet for fiscal years 1976, 1977, 1980 and 1981-1982. The mean copper levels in the infant and toddler diets for fiscal years 1977, 1980, 1981-1982 are also presented.

The grain and cereal products group; fruits and vegetables group; and meat, fish and poultry group contributed the largest percentages of copper to the diet of the adult male, accounting for 30.8, 30.1 and 21.2%, respectively, of total copper intake. In the infant diet, the meat, fish and poultry group, and the milk group accounted for 12.5 and 25.0%, respectively, of copper intake; these two food groups contributed 21.1 and 7.0%, respectively, to the toddler diet (Pennington et al., 1984).

Air. Very limited information was obtained on the presence of copper in ambient air. The U.S. EPA's Environmental Monitoring Systems Laboratory provided information from a nationwide study on copper concentrations in ambient air. The data obtained from the study include samples that were collected and analyzed from 424 sites in 49 states, the District of Columbia, Puerto Rico and the Virgin Islands for the years 1977-1983 (U.S. EPA, 1984). Concentrations of copper in the 23,814 air samples analyzed had an overall range of 0.003-7.32 $\mu g/m^3$, with a range of median and mean values by city and by year of 0.004-1.79 and 0.0043-1.96 $\mu g/m^3$, respectively.

Summary

Table IV-2 presents a general view of the total amount of copper received by a 70 kg adult male from ambient air, food and drinking water.

TABLE IV-1

Copper Levels in Adult Male, Infant and Toddler Diets by Fiscal Year*

Fiscal Year	Mean Intake (mg/day)		
	Adult	Infant	Toddler
1976	1.5	0.58	0.76
1977	1.5	0.54	0.65
1980	1.6	0.56	0.68
1981-1982	1.6	--	--

*Source: Pennington et al., 1984

TABLE IV-2

Estimated Intake of Copper from the Environment by Adult Males in µg/kg/day

Concentration in Drinking Water (mg/ℓ)	Estimated Total Intake in µg/kg/day from Drinking Water, Food and Air (% from drinking water):
0.0	22 (0%)
0.01	22 (1.3%)
1.0	51 (57%)
2.0	79 (72%)
10	310 (94%)
50	1400 (100%)

Intake from each source:

Water: 0.01 mg/ℓ: 0.29 µg/kg/day
1.0 mg/ℓ: 29 µg/kg/day
2.0 mg/ℓ: 57 µg/kg/day
10 mg/ℓ: 290 µg/kg/day
50 mg/ℓ: 1400 µg/kg/day

Air: 1.79 µg/m³: 0.59 µg/kg/day

Food: 1.5 mg/day: 21 µg/kg/day

Six separate exposure levels in drinking water, one exposure level in foods and one exposure level from ambient air are shown in the table.

The data presented have been selected from an infinite number of possible combinations of concentrations for the three sources. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to copper at any of the combined exposure levels. The data presented represent possible exposures based on the occurrence data and the estimated intakes.

The drinking water levels selected are representative of the range of levels observed in the studies obtained. It is assumed that the 70 kg adult male consumes 2 ℓ/day of drinking water.

The dietary copper intake level is representative of the level (1.5 mg/day) observed in the composite diet studies of Pennington et al. (1984).

The ambient air intake level was determined by using the high value of the range of median levels reported in ambient air (see Air Section). There was essentially no difference in the contribution of ambient air to total exposure from copper by calculating total intake using the low value of the range of median levels. It is assumed here that the average adult male inhales 23 m^3 of air/day (ICRP, 1975). Assuming an intake of 0.59 μg/kg/day from air and the food intake of 1.5 mg/day, drinking water would be the predominant source of copper exposure in the adult male when drinking water levels exceed ~0.75 mg/ℓ.

An accurate assessment of the number of individuals for which drinking water is the predominant source of exposure cannot be determined from the data since specific locations containing high concentrations of copper in drinking water and low concentrations of copper in ambient air and food are unknown.

The relative source contribution data are based on estimated intake and do not account for a possible differential absorption rate for copper by route of exposure. In addition, the effects of the chemical on the body may vary by different routes of exposure.

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V. HEALTH EFFECTS IN ANIMALS

General Toxicity

Copper is regarded as an essential element in mammalian nutrition because it is required in many enzymatic reactions. However, cells that lack barriers to copper absorption are quite susceptible to its toxic effects. Copper can inhibit the catalytic activity of many enzymes, which may be related to inhibition of sulfhydryl groups (Walsh and Crosson, 1977). Copper deficiency can also result in decreased iron absorption and iron deficiency as a result of the interaction of copper, zinc and iron (NAS, 1977). The ratio of these metals appears to be as important as the absolute quantity in the development of symptoms. In an early study, Boyden et al. (1938) reported that rats require ~50 μg/day of copper to maintain health.

Chronic copper administration to animals has been demonstrated to cause hepatic and renal cell degeneration, hemoglobinurea, jaundice and early death. Single-celled organisms, fish and ruminant animals appear to be particularly sensitive to the toxic effects of copper.

Copper plays an important role in mammalian reproduction, as deficiency of this metal leads to reproductive abnormalities. Some evidence exists to implicate copper as an animal teratogen when present in excess quantities.

Acute Toxicity. Boyden et al. (1938) studied the effects of feeding high levels of copper to albino rats for 4 weeks. Groups of 3-5 mixed male and female 28-day-old albino rats were maintained on diets supplemented with 0, 500, 1000, 2000 or 4000 ppm copper (as copper sulfate) for 4 weeks. The authors stated that these dosage levels resulted in average copper intakes of 0, 5.1, 8.2, 10.8 and 7.6 mg/rat/day based on observed food consumption.

Animal body weight was recorded weekly. All rats maintained on the 4000 ppm diet died within 1 week. Reduced body weight gain and food intake and jaundice were observed in rats maintained on diets containing 2000 ppm copper. Reduced body weight gain and food intake were observed in rats maintained on diets containing 1000 or 500 ppm copper. At the end of the 4th week of exposure, blood samples were taken, the rats were killed and their gross pathology was examined. The livers and spleens were removed for analysis of copper content. The liver had increased copper concentrations with increased copper dosage. The blood showed the most increase between 500 and 1000 ppm, that is in rats fed the diet that was toxic. Greater copper accumulation was observed in the blood, spleen and especially liver of all treated rats in comparison with the control group. The authors stated that slight toxicity was observed in rats fed 500 ppm and toxicity increased as the dietary copper level was increased (Table V-1).

Haywood (1980) observed reversible renal and hepatic toxicity in rats fed diets supplemented with a high level of copper for up to 15 weeks. Groups of four male weanling rats (strain not stated) were maintained on diets supplemented with 0 or 2000 mg Cu (as copper sulfate)/kg and killed at intervals of 1, 2, 3, 6, 9 and 15 weeks. Upon sacrifice, the livers and kidneys were removed, examined histologically, and were assayed for copper content. Animals maintained on the test diet for 1 week had normal livers and kidneys, and there was no evidence of copper accumulation in these organs. After 2 weeks of exposure, the livers of all rats on the test diet showed hypertrophy of liver parenchymal cells and accumulation of copper in the liver. Kidneys were normal and had not accumulated copper. By 3 weeks on the test diet, the livers of treated rats had clearly established areas

TABLE V-1

Toxicity Data in Rats

Species/Strain	Sex/No.	Weight/Age	Compound	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/NR	M/4	NR/weanling	$CuSO_4$	oral	diet	0 (control)	1-15 weeks	No observed effect	Haywood, 1980
						2000 mg Cu/kg diet	1 week	No observed effect	
							2 weeks	Hypertrophy of liver	
							6 weeks	Liver and kidney necrosis	
							9 weeks	Regeneration	
							5 weeks	Regeneration	
Rat/rattus rattus albino	M/10	90-110 g/ 90 days	$CuSO_4$	oral	gavage (vehicle NR)	0 (control) 100 mg $CuSO_4$/kg/day	20 days 20 days	No observed liver and kidney necrosis, although body weight, tail length and hematocrit were decreased	Rana and Kumar, 1980
Rat/white	M/4, F/4 M/4, F/4	NR/21 days	$CuSO_4$	oral	diet	0 (control) (500 ppm diet) 5 mg Cu/day	27 days 27 days	No observed effect Slight growth reduction, increased blood, spleen and liver Cu concentration	Boyden et al., 1938
	M/1, F/2					(1000 ppm diet) 8.2 mg Cu/day	27 days	Reduced growth and food intake, increased blood, spleen and liver Cu concentration	Boyden et al., 1938
	M/5, F/3					(2000 ppm diet) 10.8 mg Cu/day	27 days	No growth. Increased blood, spleen and liver Cu concentration	Boyden et al., 1938
	M/1, F/2					(4000 ppm diet) 7.6 mg Cu/day	27 days	Rapid weight loss and death probably from starvation	Boyden et al., 1938
Rat/Wistar	M/4	90-100 g/ "young"	$CuSO_4$	oral	diet	none added (control; 5.1 ppm Cu)	35 days	4.3 µg Cu/g wet liver	Miranda et al., 1981
						50 ppm added (55.1 ppm Cu)		67.1 µg Cu/g wet liver significant at $p<0.05$	Miranda et al., 1981

TABLE V-1 (cont.)

Species/Strain	Sex/No.	Weight/Age	Compound	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/Albino	M/12	100-200 g/NR	$CuCl_2$	i.p.	saline	0 (0.5 mℓ saline)	21 days	Baseline enzymatic activities	Malhotra et al., 1982
						2 mg Cu/kg/day		Brain ATPase, SDH, Cytochrome C-oxidase, Diaphorase, MDH, and 5-hydroxytryptamine not significantly different from controls	Malhotra et al., 1982
								Levels of brain Dopamine and norepinephrine significantly ($p<0.05$) higher than controls	Malhotra et al., 1982
Rat	NR	NR	copper acetate	oral	diet	250 ppm Cu acetate diet	16 months	Renal and hepatic copper accumulation	Howell, 1959

NR = Not reported

of hypertrophied hyperchromatic parenchymal cells, some of which were necrotic. Marked liver deposition of copper was observed. Proliferative activity in the kidneys and absence of renal copper accumulation was also observed. After 6 weeks on the test diet, all livers had marked changes, consisting of widespread and sometimes severe necrosis and bile duct hyperplasia. Some necrotic areas had macrophages present, and regenerative activity in surviving cells was observed. In the kidney, individual variability in effect was observed, but some kidneys had extensive desquamation of the epithelial cells in the proximal convoluted tubules. Some regenerative activity was observed in surviving epithelial cells. The livers of rats treated for 9 weeks had necrosis present in the periportal zone, bile duct hyperplasia and bands of fibroblastic connective tissues. Active healing with regeneration of parenchymal tissues was well established. Little deposition of copper in the liver was observed. The kidneys of rats treated for 9 weeks were undergoing regeneration of the proximal convoluted tubules, and most appeared reconstituted. Copper was present in the kidney. After 15 weeks on the test diet, advanced healing of the liver was observed, but bile duct hyperplasia and necrotic remnants were still present. In the kidney, reconstitution was mostly complete, but a low level of regenerative activity was observed. The authors discussed the triphasic nature of renal copper toxicity in the rat. The first phase was characterized by rising copper content with little initial effect, but increased cellular disruption with increasing concentration. When copper content reached a maximum, a second phase characterized by severe necrosis was observed. A third regenerative phase was then observed and was characterized by declining liver copper content and repair of damaged tissue.

Rana and Kumar (1980) reported liver and kidney necrosis in rats given copper sulfate by gavage for 20 days. Groups of 10 male albino rats, (Rattus rattus albino) 90 days of age were given 0 or 100 mg copper sulfate/kg bw/day by gavage (vehicle not reported) for 20 days. Based on the molecular weight of copper/copper sulfate, the amount of copper given each day was 0 or 25.4 mg/kg. Body weights and tail lengths (a measure of skeletal growth) were recorded each day. On day 20, blood samples were drawn for evaluation of red blood cell count, white blood cell count, hemoglobin level and hematocrit percentage. All animals were then killed, and their livers and kidneys were removed for wet weight measurement and routine histological examination. Several changes in treated rats, absent in control rats, were observed upon gross examination. The paws of treated rats had changed from pink to white. Significant decreases in the tail length, body weight gain, hemoglobin concentration, hematocrit percentage and red blood cell count of treated rats were observed as compared with controls. Upon histological examination, the livers and kidneys of control rats appeared normal. In treated rats, however, copper-induced damage to these organs was observed. The livers of treated rats showed centrilobular necrosis, perilobular sclerosis and heavy deposition of copper in centrilobular parenchyma. Necrosis, tubular engorgement and copper retention were observed in the kidneys of treated rats.

Malhotra et al. (1982) reported that i.p. injection of cupric chloride (2 mg Cu/kg) produced neurological changes in rats. Groups of 12 ITRC bred male albino rats received daily injections of saline or copper for 21 days. Brain mitochondrial enzyme levels were determined on these rats, and no effect of copper was observed on the levels of cytochrome-c-oxidase, malate dehydrogenase, diaphorase, succinic dehydrogenase, adenosine triphosphatase or 5-hydroxytrypt-amine. Statistically significant ($p<0.05$) increases were observed in brain dopamine and norepinephrine levels in treated rats when compared with controls.

In a series of studies on pigs, Suttle and Mills (1966a,b) demonstrated that relatively low levels of copper salts in the diet can produce adverse effects depending on the diet. Groups of six Large White female pigs were maintained on maize meal diets containing 750 ppm of basic copper carbonate [$(Cu(O_3)\cdot Cu(OH)_2\cdot H_2O$] alone or with supplemental zinc (500 ppm $ZnCO_3$) or iron (750 ppm $FeSO_4\cdot 7H_2O$). Zinc and iron treatments lasted for 42 and 49 days, respectively. The copper doses to the pigs were ~22 mg Cu^{+2}/kg/day, as calculated from weight and food intake data. The control group in this experiment was the 12 pigs that received copper without zinc or iron. Severe toxic effects were noted (Table V-2), but protection was afforded by providing the nutrients iron or zinc.

In a second experiment, Suttle and Mills (1966b) maintained groups of six female Large White pigs on maize meal diets supplemented with 600 ppm basic copper carbonate to which soybean meal, dried skim milk or white fish meal protein supplements were added. The treatment lasted for 48 days and the pigs in the soya, milk and fish fed groups received 6.4, 11.0 and 15.4 mg Cu^{+2}/kg/day, respectively. Gradual development of anemia was observed in all treatment groups, but the fish fed group had signs of toxicity (see Table V-2). It was presumed that the high calcium levels in the fish meal diet adversely affected zinc availability, and thus led to toxicity.

TABLE V-2

Acute and Subchronic Oral Toxicity Data in Pigs

Species/Strain	Sex/No.	Age	Compound	Vehicle	Dose	Duration (days)	Effects	Comments	Reference
Pig/Large White	F/12	weanling	copper carbonate	maize diet	750 ppm diet: -22 mg Cu^{+2}/kg/day	49	Increased serum copper and aspartate transaminase (AST) in 9/12, and jaundice in 7/12. Reduced hemoglobin. Centrilobular necrosis and bile canaliculi disruption	Addition of iron (500 ppm) and zinc provided protection	Suttle and Mills, 1966a
Pig/Large White	F/6	weanling	copper carbonate	maize and soya diet	600 ppm diet: 6.4 mg Cu^{+2}/kg/day	48	Gradual development of anemia		Suttle and Mills, 1966b
Pig/Large White	F/6	weanling	copper carbonate	maize and skim milk diet	600 ppm diet: 11.0 mg/kg/day	40	Gradual development of anemia		Suttle and Mills, 1966b
Pig/Large White	F/6	weanling	copper carbonate	maize and fish meal diet	600 ppm diet: 15.4 mg/kg/day	48	Increased serum copper, increased AST growth rate reduction. Gradual development of anemia and jaundice	High calcium in fish may have affected Zn availability	Suttle and Mills, 1966b
Pig/Large White	F/6	weanling	copper sulfate·5H$_2$O	maize and fish meal diet	250 ppm diet: 2.6 mg Cu^{+2}/kg/day	79	Slight weight gain, increased liver copper concentrations, increased AST, jaundice	High calcium in fish may have affected Zn availability	Suttle and Mills, 1966b
Pig/Large White	F/6	weanling	copper sulfate·5H$_2$O	maize and fish meal diet	425 ppm diet	47-60	Severe growth depression and toxicosis after 14 days. Gastrointestinal hemorrhages	High calcium in fish may have affected Zn availability	Suttle and Mills, 1966b
Pig/Hampshire and Yorkshire	NR/8	NR	copper sulfate·5H$_2$O	corn-soy diet	250 ppm diet 3.2 mg Cu^{+2}/kg/day	61	Accelerated growth with less feed		Kline et al., 1971

TABLE V-2 (cont.)

Species/Strain	Sex/No.	Age	Compound	Vehicle	Dose	Duration (days)	Effects	Comments	Reference
Pig/Hampshire and Yorkshire	NR/8	NR	copper sulfate$\cdot 5H_2O$	corn-soy diet	500 ppm diet 5.5 mg Cu^{+2}/kg/day	61	Reduced growth and hemoglobin levels, increased liver copper concentrations		Kline et al., 1971
Pig/Hampshire and Yorkshire	NR/12	NR	copper sulfate$\cdot 5H_2O$	corn-soy diet	0 supplemental Cu	88	Normal hemoglobin hematocrit and liver copper levels		Kline et al., 1971
Pig/Hampshire and Yorkshire	NR/12	NR	copper sulfate$\cdot 5H_2O$	corn-soy diet	150 ppm diet supplement; 1.8 mg Cu^{+2}/kg/day	88	Accelerated weight gain		Kline et al., 1971
Pig/Hampshire and Yorkshire	NR/12	NR	copper sulfate$\cdot 5H_2O$	corn-soy diet	200 ppm diet supplement; 2.5 mg Cu^{+2}/kg/day	88	Accelerated weight gain		Kline et al., 1971
Pig/Hampshire and Yorkshire	NR/12	NR	copper sulfate$\cdot 5H_2O$	corn-soy diet	250 ppm diet supplement; 2.9 mg Cu^{+2}/kg/day	88	Accelerated weight gain		Kline et al., 1971

NR = Not reported

Suttle and Mills (1966b) then maintained groups of six female Large White pigs on maize meal/white fish diets containing 0, 250 or 425 ppm of copper sulfate ($CuSO_4 \cdot 5H_2O$) for 60-79 days. The group treated with 425 ppm copper sulfate became so intoxicated that euthanasia was performed on 4/6 by the 60th day. The 250 ppm (2.6 mg Cu^{+2}/kg/day) group had slight weight gain for the first 30 days of treatment, but 3/6 had jaundice by the 46th day. Serum copper and aspartate transaminase levels were significantly increased compared with controls. On the 79th day, the hepatic copper concentration of treated pigs was significantly increased compared with controls.

Kline et al. (1971) maintained groups of eight pigs on a corn-soy diet supplemented with 250 (3.2 mg Cu^{+2}/kg/day) or 500 (5.5 mg Cu^{+2}/kg/day) ppm copper sulfate for 61 days. In a second experiment, groups of 12 pigs were fed a corn-soy diet supplemented with 0, 150 (1.8 mg Cu^{+2}/kg/day), 200 (2.5 mg Cu^{+2}/kg/day) or 250 (2.9 mg $Cu^{+}2$/kg/day) ppm copper sulfate for 88 days. Animals were weighed and food consumption was recorded. Blood samples were drawn and representative animals were slaughtered at the end of treatment. The control group was normal and had normal blood parameters. Pigs fed 150-250 ppm copper sulfate (1.8-3.2 mg Cu^{+2}/kg/day) all had accelerated weight gain but also had increases in liver copper that indicated a significant ($p<0.05$) linear correlation with dose. Reduced growth and hemoglobin levels and increased liver copper were observed in the 500 ppm treatment group.

Chronic and Subchronic Toxicity. Effects of feeding copper sulfate to pigs for a subchronic duration (88 days) were previously discussed in the Acute Toxicity Section.

There are limited data regarding the chronic toxicity of copper in laboratory animals. Only one study (Howell, 1959) was available, and the emphasis of this study was on copper distribution rather than on a careful examination of toxicologic endpoints.

Howell (1959) maintained rats on diets supplemented with 5000 ppm copper acetate for 16 months. This dose is equivalent to 250 mg Cu acetate/kg/day (79.6 mg Cu/kg/day), assuming that rats consume food at a rate of 5% of their body weight per day. No copper deposition was found in the brain or cornea. Liver and kidney tissues were observed to heavily accumulate copper.

Target Organ Toxicity

Copper is widely distributed in all human tissues. In cases of abnormal copper metabolism such as Wilson's disease or when copper is absorbed in excessive amounts, the metal can accumulate in various tissues and exert a toxic effect. Experimental studies have often been designed to examine in histological detail those target organ effects observed symptomatically in humans.

Liver. The liver is the main storage depot for copper, and the damage done in this organ is associated with accumulation of the metal. The specific etiology of copper-induced liver damage has not been completely determined (Bremner, 1979). Administration of copper compounds to laboratory animals has resulted in hepatocellular necrosis, regenerative activity, cirrhosis, Kupffer cell mobilization and hepatocellular pigment formation (Barka et al., 1964). Chuttani et al. (1965) reported the presence of centrilobular necrosis upon conducting autopsies on suicide victims who ingested copper sulfate.

Kidney. Kidney damage is associated with copper toxicosis. Excessive ingestion of copper was reported to cause renal tubular damage (Walsh and Crosson, 1977) and renal failure (Sanghvi et al., 1957) upon acute exposure in humans and kidney necrosis in rats upon subchronic administration (Rana and Kumar, 1980; Haywood, 1980). Chuttani et al. (1965) reported hematuria, hemoglobinuria, high blood urea nitrogen and oliguria as typical symptoms in attempted suicides involving copper sulfate. Kidney biopsies in these cases demonstrated that severe renal lesions had occurred, including congestion of glomeruli and necrosis.

Haywood (1980) reported that the onset of kidney necrosis in rats occurred only after the liver began to accumulate high levels of copper. Bremner and Davies (n.d.) gave i.v. injections of copper thionein to rats and observed renal copper thionein accumulation and signs of kidney damage. These studies lend support to the unconfirmed hypothesis that copper thionein leaks from the liver of copper-poisoned animals and is responsible for the subsequent renal damage (Bremner, 1979).

Blood. Several investigators have described a sudden hemolytic crisis in sheep poisoned by copper. Humans have suffered from hemolytic anemia caused by Wilson's disease (Scheinberg and Sternlieb, 1969), ingestion of large amounts of copper (Roberts, 1956; Walsh et al., 1977) and absorption of copper from kidney dialysis machines using copper components (Blomfield et al., 1971).

Moroff et al. (1974) incubated human erythrocytes for 4 hours in a 1 mM copper sulfate solution. Approximately 5-10% of the erythrocytes underwent hemolysis, and significant increases in osmotic fragility were observed to occur within 3 hours.

Erythrocyte glucose-6-phosphate dehydrogenase (G6PD) levels have been reported to decrease as a result of human copper sulfate poisoning (Walsh and Crosson, 1977; Chugh et al., 1975).

Lungs. Adverse effects in humans of copper inhalation include metal fume fever from exposure to fumes (Stokinger, 1980) and to finely divided copper dust (Gleason, 1968). An occupational disease, vineyard sprayers' lung, is associated with chronic inhalation exposure of Bordeaux Mixture ($CuSO_4$ and lime) aerosol. Pulmonary copper deposition and resultant lesions in vineyard sprayers were first described by Pimental and Marques (1969) and characterized by Pimental and Menezes (1975). Histiocytic granulomas, fibrohyaline nodular scars and tumors are typical lesions that have been observed. However, these workers are also exposed to arsenic.

Pimental and Marques (1969) reproduced lesions characteristic of vineyard sprayers' lung in guinea pigs exposed to saturated atmospheres of Bordeaux Mixture. Groups of six guinea pigs were exposed to air or the smoke from burning sulfur wicks (3 times/day) for 6.5 months. A group of 12 guinea pigs were exposed 3 times/day to an atmosphere saturated with Bordeaux Mixture for 6.5 months. The pathology observed in the guinea pigs exposed to the mixture was similar to that observed in humans exposed to Bordeaux Mixture. Copper-containing micronodular lesions and small histiocytic granulomas were among the lesions observed in exposed guinea pigs.

Gastrointestinal Tract. In humans, a metallic taste, nausea, vomiting and epigastric pain are the first symptoms reported in nearly all cases of acute poisoning with copper sulfate (Chuttani et al., 1965). Copper sulfate has been used medically as an emetic at a dose of 300 mg (Hoover, 1970), and ingestion of as little as 5.3 mg of copper has been reported to cause emesis (Wyllie, 1957).

Gastrointestinal hemmorhages were observed in copper-poisoned pigs (~5 mg Cu^{+2}/kg/day) (Suttle and Mills, 1966b).

Central Nervous System. Chronic copper toxicosis in Wilson's disease is associated with accumulation of the metal and pathological changes in the brain. When liver cells are damaged by the excessive concentrations of copper they store, copper is released and can accumulate in the CNS. Untreated patients have developed tremors, seizures, psychoses and behavioral disorders.

While copper has been reported to be acutely toxic when injected directly into the subarachnid space of animals, acute copper poisoning does not result in increased brain copper levels (Owen, 1981). Malhotra et al. (1982) reported increases in brain dopamine and norepinephrine in rats given an i.p. injection of 2 mg/kg/day for 21 days. Sharma and Obersteiner (1981) observed moderate cytotoxicity of copper sulfate (10^{-4} to 10^{-6} M Cu) when incubated with chick ganglia cultures.

Other Effects

Carcinogenicity. BRL (1968) studied the carcinogenicity of a copper-containing compound, copper hydroxyquinoline, in two strains of mice (B6C3F1 and B6AKF1) fed a diet that provided sufficient copper (i.e., 5.7 mg Cu/kg feed). The copper complex was administered orally and by subcutaneous injection. Using subcutaneous administration, groups of 18 male and 18 female 28-day-old mice of both strains were given a single injection of gelatin or 1000 mg copper hydroxyquinoline/kg bw (180.6 mg Cu/kg) suspended in 0.5% gelatin. The animals were observed until they were 78 weeks old, and then killed. Oral exposure consisted of similar groups of 7-day-old mice treated daily by gavage with 1000 mg copper hydroxyquinoline/kg bw (i.e., 180.6 mg Cu/kg) suspended in 0.5% gelatin until age 28 days, whereupon the compound was administered in the feed at a concentration of 2800 ppm (505.6 ppm Cu). Animals were fed the treated diet until they were 78 weeks old, at which time they were killed. Positive, negative, vehicle and untreated control animals were also maintained and compared with treated animals. All animals killed or found dead were subjected to routine macro- and microscopic histological analysis to identify tumor-bearing tissues. No statistically significant increases (with respect to controls) in the incidence of lymphatic leukemias, reticulum cell sarcomas, pulmonary adenomas or carcinomas, hepatomas, hepatic carcinomas, mammary carcinomas, skin carcinomas or cavernous angiomas were observed in orally-treated mice.

In the portion of the study using subcutaneous exposure, male B6C3F1 mice had an increased incidence of reticulum cell sarcomas compared with controls (e.g., 6/17 treated; 8/141 control; $p<0.001$). No tumors were observed in treated male B6AKF1 mice. Female mice of either strain had low incidences of reticulum cell sarcomas. Treated and control B6C3F1 females had incidences of reticulum cell sarcoma of 1/18 and 1/154, respectively. Treated and control B6AKF1 females had incidences of reticulum cell sarcoma of 3/18 and 5/157, respectively (BRL, 1968).

Gilman (1962) studied the carcinogenicity of cupric oxide, cupric sulfide and cuprous sulfide in 2- to 3-month-old Wistar rats. Groups of 30-32 rats were given single intramuscular injections containing 20 mg of cupric oxide (16 mg Cu), cupric sulfide (13.3 mg Cu) and cuprous sulfide (16 mg Cu) into the left and right thigh of each rat. All animals were observed for up to 20 months, after which histopathological evaluation was conducted. Controls were not reported. Of the animals receiving cupric oxide, cupric sulfate and cuprous sulfate, the ratios of animals surviving the experiment/animals dosed were 10/32, 19/30 and 18/30, respectively. No injection-site tumors were observed, and the groups of animals receiving cupric oxide, cupric sulfide and cuprous sulfide had 0, 2 and 1 tumors, respectively.

Haddow and Horning (1960) published a table of bioassay results on various copper compounds from which Table V-3 was prepared. No other experimental detail was provided.

Mutagenicity. The available data obtained from *in vitro* mutagenicity assays do not provide sufficient evidence to form a conclusion regarding the mutagenicity of copper (Table V-4).

TABLE V-3

Tumorigenicity of Some Copper Compounds*

Agent Under Test	Number and Strain of Mice	Number of Weekly Subcutaneous Injections/Dose	Months of Experiment to Date and Survivors	Tumors Recorded
Copper-dextran	20 stock	6/0.1 cc of 1 in 4 dilution	10 (13)	None
8-hydroxyquinoline copper complex	20 stock	39/0.1 mg	10 (14)	1 pleomorphic sarcoma
Cross-conjugated macrocycle copper porphyrin	20 stock	4/0.5 mg	10 (14)	None
Copper phthalocyanine	20 stock	34/0.5 mg	8 (17)	None
Copper phthalocyanine tetra-3-sulfonic acid	20 stock	36/0.5 mg	8 (20)	None
Copper phthalocyanine tetra-4-sulfonic acid	20 stock	25/0.5 mg	8 (11)	None

*Source: Haddow and Horning, 1960

TABLE V-4

Mutagenicity Data for Copper Compounds

Assay	Indicator/ Organism	Application	Concentration or Dose	Activating System	Response*	Comment	Reference
Reverse mutation	*Salmonella typhimurium* TA98, TA100	plate incorporation	≤5000 μg copper sulfate/plate	± rat liver S-9	-	NC	Moriya et al., 1983
Reverse mutation	*S. typhimurium* TA100	plate incorporation	copper 8-quinolinolate 5-10 μg/plate	+ rat liver S-9	+	NC	Moriya et al., 1983
Reverse mutation	*S. typhimurium* TA100	plate incorporation	0.5-10 μg copper 8-quinolinolate/plate	- rat liver S-9	-	NC	Moriya et al., 1983
Reverse mutation	*S. typhimurium* TA98, TA1535, TA1537, TA1538	plate incorporation	≤5000 μg copper 8-quinolinolate/plate	± rat liver S-9	-	NC	Moriya et al., 1983
Reverse mutation	*S. typhimurium* TA100, LT2	spot test (paper disc)	10 μℓ of 10^{-6} to 10^{-1} M aqueous solution of $CuCl_2 \cdot 2H_2O$	NA	-	NC	Tso and Fung, 1981
Reverse mutation	*Saccharomyces cerevisiae* D-7	spot test (center well)	0.1 M copper sulfate	NA	-	NC	Singh, 1983
Reverse mutation	*Escherichia coli* WP2 hcr	plate incorporation	≤5000 μg copper 8-quinolinolate/plate	NA	-	NC	Moriya et al., 1983
Reverse mutation	*E. coli* Sd-4	plate incorporation	2-10 ppm copper sulfate	NA	+	Dose related at ≥2 ppm	Demerec et al., 1951
Growth inhibition (rec)	*Bacillus subtilis* H17, M45	spot test (paper disc)	0.05 mℓ of 0.05 M CuCl or $CuCl_2$ solution	NA	-	NC	Nishioka, 1975
Growth inhibition (rec)	*B. subtilis* M16 17, M16 45	liquid cultivation	16.5-18 mg copper sulfate/ℓ	NA	-	NC	Matsui, 1980
Growth inhibition (rec)	*B. subtilis* H17, M45	spot test (paper disc)	0.05 mℓ of 0.001-10 M CuCl or $CuCl_2$ solution	NA	-	NC	Kanematsu et al., 1980
Chromosome aberrations	Avian myeloblastosis virus, DNA polymerase	liquid holding	20-150 mM $CuCl_2$ or $Cu(C_2H_3O_2)_2$	NA	+	NC	Sirover and Loeb, 1976

TABLE V-4 (cont.)

Assay	Indicator/ Organism	Application	Concentration or Dose	Activating System	Response*	Comment	Reference
Chromosome aberrations	rat hepatocytes	plate incorporation, then alkaline elution	0.03-0.3 mM cupric sulfate	NA	-	Elution rate ≤3 times the control rates	Sina et al., 1983
Chromosome aberrations	rat hepatocytes	plate incorporation, then alkaline elution	1.0 mM cupric sulfate	NA	+	Elution rate ≥3 times controls rates	Sina et al., 1983
Cell transformation	Syrian hamster embryo cells by simian adenovirus SA7	plate incorporation	≥0.38 mM Cu_2S	NA	+	Enhancement ratio: $\frac{\text{Transformation frequency treated}}{\text{Transformation frequency control}}$ = 16.2	Casto et al., 1979
Cell transformation	Syrian hamster embryo cells by simian adenovirus SA7	plate incorporation	0.08-0.64 mM $CuSO_4$	NA	+	Enhancement ratio = 2.2	Casto et al., 1979
Recessive lethals	*Drosophila melanogaster* Oregon-R	microinjection into larvae	0.1% $CuSO_4$	NA	+	1.06% lethals (0% in controls)	Law, 1938
Recessive lethals	*D. melanogaster* Oregon-R	immersion of eggs for 10 minutes	concentrated aqueous solution of $CuSO_4$	NA	+	1.25% lethals (0% in controls)	Law, 1938
Mitotic abnormalities	MTK-sarcoma III rat ascites	*in vivo*	150 mg copper sulfide/kg i.p.	NA	+	Chromatic aggregation, stickiness, contraction, scattering, lagging and clumping of chromosomes	Kimura and Makino, 1963
Mitotic abnormalities	MTK-sarcoma III rat ascites	*in vivo*	300 mg copper sulfate/kg i.p.	NA	+	Reversible events: lobated nuclei, karyorrhexis and multipolar spindle formation	Kimura and Makino, 1963

NA = Not applicable; NC = No comment

A reverse mutation assay reported dose-related mutation in _E_. _coli_ with 2-10 ppm copper sulfate (Demerec et al., 1951). More recently, Moriya et al. (1983) reported the absence of mutation in _E_. _coli_ incubated with up to 5 mg copper quinolinolate/plate and in _Salmonella_ _typhimurium_ strains TA98, TA1535, TA1537 and TA1538. Copper 8-quinolinolate was mutagenic to _S_. _typhimurium_ strain TA100, but only when a source of mammalian metabolic activation was included (Moriya et al., 1983). Up to 5 mg of copper sulfate/plate did not induce reverse mutations in _S_. _typhimurium_ TA98 and TA100 either with or without metabolic activation.

Other investigators have obtained negative mutagenic results with copper sulfate or copper chloride in other microbial assays. These include _Saccharomyces_ _cerevisiae_ D-7 (Singh, 1983) and _Bacillus_ _subtilis_ (Nishioka, 1975; Matsui, 1980; Kanematsu et al., 1980).

Several isolated cell mutagenicity assays have produced positive results with copper compounds. Errors in DNA synthesis from poly(c)templates have been induced in viruses (Sirover and Loeb, 1976) and chromosome aberrations have resulted in isolated rat hepatocytes (Sina et al., 1983) when incubated in 20-150 mM $CuCl_2$ or $Cu(C_2H_3O_2)_2$ and 1.0 mM $CuSO_4$, respectively. Casto et al. (1979) induced enhanced simian adenovirus cell transformation in Syrian hamster embryonic cells with the addition of 0.38 mM Cu_2S and to a lesser extent with 0.08 mM of $CuSO_4$.

High concentrations of copper compounds have been reported to induce abnormalities at mitosis in rat ascites cells and recessive lethals in _Drosophila melanogaster_. Law (1938) reported increases in the percent lethals observed in Drosophila larvae and eggs when exposed to copper by microinjection (0.1% $CuSO_4$) or immersion (concentrated aqueous $CuSO_4$), respectively.

Teratogenicity and Reproductive Toxicity. Copper deficiency has been observed to produce teratogenic response in lambs, goats, rats, guinea pigs, dogs and chicks. Terata include neural degeneration, reduced growth, skeletal malformations and cardiovascular lesions (Hurley and Keen, 1979).

The spermicidal properties of copper are well known and were first demonstrated in the 19th century (Holland and White, 1982). Prevention of mammalian embryogenesis because of the small amounts of copper absorbed from intrauterine loops or wires fashioned from copper has been demonstrated (Oster and Salgo, 1977; Hurley and Keen, 1979).

The embryotoxicity and teratogenicity of i.v. injection of copper salts was first demonstrated in hamsters by Ferm and Hanlon (1974). Copper sulfate and copper citrate dissolved in demineralized water were both observed to reduce embryonic viability and produce abnormal offspring when injected into the lingual vein of pregnant dams on the 8th day of gestation. Day 1 of gestation was considered the day after which mating occurred. Administration of demineralized water alone produced no abnormal embryos (Table V-5). Administration of copper sulfate (2.13 mg Cu/kg) to 16 dams caused the formation of 12 abnormal of 155 live embryos (five thoracic wall hernias, four encephalocoeles, two spina bifida and one microphthalmia).

TABLE V-5

Teratogenicity Data for Copper Compounds

Compound, Route	Species/ Strain	No. Dams at Start	Vehicle	Daily Dose or Exposure	Treatment Days[a]	Observation Day	Maternal Response	Fetal Response			Reference
								Avg. Litter Size	Avg. Weight (g)	Malformations	
$CuSO_4$, oral	mouse/ C57BL	21	diet	0	30 to 0	19	NR	3.1	1.1	0	Lecyk, 1980
		10		25.9 mg Cu/kg/day[b]				4.6	1.3	0	
		18		51.7 mg Cu/kg/day[b]				4.5	1.2	0	
		7		77.6 mg Cu/kg/day[b]				4.4	1.1	0	
		10		103.5 mg Cu/kg/day[b]				4.2	1.2	0	
		22		155.3 mg Cu/kg/day[b]				2.5	1.0	1 skeletal	
		18		207.1 mg Cu/kg/day[b]				1.9	1.0	3 hernia, hydrocephalus, skeletal	
$CuSO_4$, oral	mouse/DBA	17	diet	0	30 to 0	19	NR	4.5	1.0	0	Lecyk, 1980
		10		25.9 mg Cu/kg/day[b]				5.4	1.2	0	
		10		51.7 mg Cu/kg/day[b]				5.1	1.2	0	
		14		77.6 mg Cu/kg/day[b]				4.1	1.2	0	
		10		103.5 mg Cu/kg/day[b]				4.1	1.1	0	
		18		155.3 mg Cu/kg/day[b]				3.1	1.1	2 skeletal	
		20		207.1 mg Cu/kg/day[b]				2.7	1.1	4 encephaloceles, skeletal	
Copper citrate, i.p.	hamsters/ Golden	37	D.I. H_2O	0	8	12-13	NR	Free of gross teratogenic effects (0/455), 2/37 had abnormalities, 2/68 heart defects			DiCarlo, 1980
		89	D.I. H_2O	27 mg/kg			No effect on survival, but a 10% reduction in body weight gain.	45/855 fetuses with gross defects (limb, tail, edema) 19/89 litters had abnormalities, 58 ventricular septal defects in 49 fetuses.			
$CuSO_4$, i.v.	hamsters/ Golden	10	D.I. H_2O	0	8	12-13	NR	92% viable embryos, 8% resorption, 0% abnormal			Ferm and Hanlon, 1974
		16		2.13 mg Cu/kg			NR	74% viable embryos, 26% resorption, 6% abnormal			
		3		4.25 mg Cu/kg			NR	14% viable embyros, 86% resorption, 8% abnormal			
		3		7.50 mg Cu/kg			NR	0% viable embryos, 74% resorption, 8% abnormal			
		2		10.0 mg Cu/kg			Death				

TABLE V-5 (cont.)

Compound, Route	Species/ Strain	No. Dams at Start	Vehicle	Daily Dose or Exposure	Treatment Days^a	Observation Day	Maternal Response	Fetal Response	Reference
Copper citrate, i.v.	hamsters/ Golden	13	D.I. H_2O	0.25-1.5 mg Cu/kg	8	12-13	NR	83% viable embryos, 16% resorptions, 2% abnormal	Ferm and Hanlon, 1974
		6		1.8 mg Cu/kg			NR	59% viable embryos, 41% resorptions, 17% abnormal	
		8		2.2 mg Cu/kg			NR	66% viable embryos, 34% resorptions, 35% abnormal	
		2		4.0 mg Cu/kg			Death		

[a] Relative to day of conception (day 0)

[b] Assume mice consume 13% of bw/day

NR = Not reported

Similar administration of copper sulfate (at 4.25 mg Cu/kg) to three dams caused the development of four abnormal of seven live embryos (one exencephaly, one hydrocephalus, one abdominal hernia and one abnormal spinal curvature). Administration of higher doses of copper sulfate (7.5 and 10 mg/kg) resulted in 100% mortality of embryos and dams, respectively.

Copper administered in a chelated form (copper citrate) was observed to be a more potent teratogen than the uncomplexed form (copper sulfate). Administration of 0.25-1.5 mg Cu/kg (as citrate) to 13 dams resulted in the development of four abnormal of 172 live embryos (two tail defects, one microphthalmia and one craniorachischisis). Similar administration of 1.8 mg Cu/kg to six dams produced 14 abnormal of 81 live embryos (13 tail defects and 1 meningocoele). Administration of 2.2 mg Cu/kg to eight dams produced 35 abnormal embryos (25 tail defects, 6 thoracic wall defects, 2 microphthalmias, 1 abdominal wall defect and 1 facial cleft). Administration of 4.0 mg Cu/kg to two dams resulted in the death of both.

Experiments with ^{64}Cu nitrate injected into pregnant dams showed that with 0.55, 12.8, 0.53, 1.47 and 0.81 μg ^{64}Cu/g tissue in the maternal blood, maternal liver, uterus, placenta and embryo, respectively, copper permeates the hamster placenta (Ferm and Hanlon, 1974).

DiCarlo (1980) produced terata in hamsters by i.p. injection of copper citrate. Pregnant female Golden hamsters were given an i.p. injection of demineralized water either alone or containing 2.7 mg copper citrate/kg on the 8th day of gestation. The day after mating was considered day 1 of gestation. The control and dosed groups consisted of 37 and 89 animals, respectively. All dams were killed on the 12th or 13th day of gestation, whereupon all viable embryos were removed for histopathological analysis. Copper was not observed to affect maternal survival, but did reduce maternal body weight gain, possibly by inducing a high resorption rate. Control embryos were observed to be free of gross teratological effects, but on histopathological examination, 2 of 68 randomly-chosen embryos were observed to have cardiac muscular ventricular septal defects. Of the treated embryos, 45 of 855 embryos examined had gross malformations (e.g., limb and tail defects and edema). On histopathological examination, 58 cardiac defects (various ventricular septal malformations) were observed in 49 embryos with gross malformations. The author stated that copper's role as a prosthetic group in oxidative enzymes could lead to teratogenesis when present in excessive amounts by interfering with these metabolic reactions during organogenesis. However, the relevance of these studies is questionable because i.p. ingestion of a high level of copper does not duplicate any of the means whereby toxic levels might enter the body.

Lecyk (1980) oberved teratogenic effects in two strains of mice fed diets supplemented with copper sulfate before mating. Various numbers (see Table V-5) of C57BL and DBA mice were maintained for 1 month on diets supplemented with 0, 500, 1000, 1500, 2000, 3000 and 4000 ppm copper sulfate. These concentrations are equivalent to 0, 199, 398, 597, 796, 1195 and 1593 ppm Cu, respectively. Assuming that mice consume food at a rate of 13% of their body weight per day, doses at 199 and 398 ppm copper are equivalent to 25.9 and 51.7 mg Cu/kg/day, respectively. After 30 days of treatment, the females were mated with males of respective strains and the day on which a vaginal plug was observed was determined as day 0 of gestation. Pregnant mice were allowed to gestate until the 19th day, at which time they were killed and the fetuses were examined for morphological defects. Low doses (500-1000 ppm) of copper were observed to stimulate embryonic development; increased litter size and increased fetal weight resulted. Higher copper doses (>1000 ppm) increased fetal mortality and decreased litter size. When supplemented in the diet at 3000 and 4000 ppm, copper sulfate caused a level (2-8% of living fetuses) of various skeletal and other malformation that was absent at lower doses and controls. No abnormal fetuses were observed in control groups. However, the observations by Boyden et al. (1938) imply that food intake decreases at high concentrations of copper. Therefore, the actual food intake may have been seriously reduced, therefore adversely affecting fetal development.

Summary

Efficient homeostatic mechanisms generally protect mammals from the adverse effects of dietary copper deficiency and excess. With the exception of ruminant animals, the chronic toxicity of orally-administered copper has not been well investigated. An inborn error in copper metabolism in humans (Wilson's disease) may be the only form of chronic copper toxicosis in man.

Ingestion of 100 mg Cu/kg/day by rats for 1 week resulted in no observable effects (e.g., no liver accumulation and no adverse renal or hepatic morphology). Administration of this dose for up to 6 weeks caused severe renal and hepatic effects in rats. Further administration of copper at this dose to rats for up to 15 weeks resulted in no further damage; rather, regeneration of hepatic and renal tissues was observed (Haywood, 1980). Support for these observations was reported by Rana and Kumar (1980) who observed liver and kidney necrosis in rats fed 25.4 mg Cu/kg/day for 20 days.

Increased liver copper concentrations were observed in rats fed 16.7 mg Cu/kg/day for 27 days (Boyden et al., 1938) and 11.0 mg Cu/kg/day for 21 days (Miranda et al., 1981). Administration of higher levels of dietary copper resulted in elevated liver and splenic copper levels, growth reduction and reduced dietary intake resulting in death (Boyden et al., 1938).

Malhotra et al. (1982) observed that rats given daily i.p. injections of 2 mg Cu/day had significantly ($p<0.05$) elevated levels of brain dopamine and norepinephrine when compared with controls.

Pigs appear to be more sensitive than rats to the acute toxicity of copper. Suttle and Mills (1966a,b) reported adverse effects in pigs given copper supplements in doses as low as 6.4 mg Cu^{+2}/kg/day for 48 days and 2.6 mg Cu^{+2}/kg/day for 79 days. Kline et al. (1971) reported beneficial effects of copper supplementation in pigs at doses of 1.8-3.2 mg Cu^{+2}/kg/day for 61-88 days. Administration of 5.5 mg Cu^{+2}/kg/day for 61 days caused adverse effects (e.g., growth reduction, reduced hemoglobin and increased hepatic copper) (Kline et al., 1971).

Liver damage, hemolytic anemia, renal damage and gastrointestinal irritation are effects of acute copper poisoning that have been observed to occur in laboratory animals and man (Bremner, 1979; Owen, 1981).

Equivocal results have been obtained from experiments designed to evaluate the carcinogenicity and mutagenicity of copper compounds. Administration of copper compounds to mice by subcutaneous injection has been reported to induce tumor formation (BRL, 1968; Haddow and Horning, 1960). The only tumorigenicity studies for orally-administered copper were negative (BRL, 1968).

Microbial mutation assays using copper compounds have generally provided negative results. Some mutagenic activity by copper compounds at low concentrations has been observed in cell culture assays. Copper sulfate was observed to increase the frequency of recessive lethal mutations in *D. melanogaster* at high concentrations (Law, 1938).

Copper compounds have been observed to elicit a teratogenic response at ~2 mg Cu/kg when injected into female hamsters on the 8th day of pregnancy (DiCarlo, 1980; Ferm and Hanlon, 1974). Lecyk (1980) reported a teratogenic response of orally-administered copper sulfate in mice for the 30-day dietary exposures at 103.5 mg Cu/kg/day.

VI. HEALTH EFFECTS IN HUMANS

The essentiality of copper in human nutrition has long been recognized. Copper deficiency is associated with reduced hemoglobin formation, reduced elastin formation, teratogenesis, abnormal amino oxidase activity and many other problems. The literature is replete with studies regarding the nutritional essentiality of copper, but copper toxicity from chronic exposure has not been as well investigated. The NAS (1980a) has recommended daily allowance (RDA) for copper of 1.5-2.5 mg/day for a child aged 0-10 years and 2.0-3.0 mg/day for an adult aged >11 years.

Clinical Case Studies

Reports of single cases or a series of cases after exposure is believed to have occurred are useful for obtaining clinical details of adverse effects. Evidence of exposure, such as serum copper levels, support the qualitative association and help to obtain typical symptomology. However, these cases often offer limited ability to measure exposure level of the causative agent, duration of exposure to the agent, previous health status or possible exposures to other agents. Rates of response can only be obtained from planned or epidemiologic studies.

Ingestion. The potential for acute poisoning from ingestion of copper compounds has long been recognized. Several references in the pre-20th century literature describe cases of accidental human poisoning by copper compounds (Owen, 1981). The unwitting ingestion of many types of food and nonfood items containing copper has produced typical symptomatology of copper poisoning, ranging from gastrointestinal disturbances, headache, dizziness and metallic taste in the mouth, to death. In several instances the exposure is to copper sulfate; therefore, some of the toxicity may be due to the sulfate component.

Spitalny et al. (1984) and Sargent and Jean (1983) report on a family whose recurrent episodes of emesis and abdominal pain were linked to copper in their drinking water. Early morning Cu concentrations on four different days were <2, 4.8, 2.8 and 7.8 mg/ℓ. During the 1.5 year period when exposure may have been occurring, 3 of the 4 family members reported recurrent episodes of emesis and abdominal pain. Although these levels are lower that those reported in other studies of toxicity, the continuous nature of the exposure suggests that tissue Cu levels may already have been high in the family. A follow-up investigation showed that, 1) aggressive water (Langelier index -3.5) was delivered through a copper main; 2) other families receiving the same water did not show symptoms and had lower copper levels in the tap water due to a different main or location on the supply; 3) the family with symptoms had the higher copper levels in the hair than other families, these levels were greater than the normal range; 4) concentrations of arsenic, iron, lead, manganese, zinc and nitrate in the water were below EPA standards; and 5) all symptoms were resolved when the family stopped drinking the water. According to a standard approach for identifying agents that cause infectious disease, this approach helps to link copper to the illness episode. Reconstruction of quantitation exposure is more difficult, but several measures exist to suggest the range of copper concentrations associated with the episodes of illness.

Chuttani et al. (1965) studied the clinical data from 53 patients poisoned with copper sulfate in suicide attempts. The amounts of copper sulfate ingested ranged from 1 to >30 g (i.e., 0.25-7.6 g Cu). Five of the patients studied died of acute copper sulfate poisoning (specific causes unknown), and the remaining 48 exhibited a variety of symptoms (Table VI-1). All the patients studied had a metallic taste in their mouths, nausea, vomiting and epigastric pain. Levels of serum total copper, serum ionic copper and whole blood copper were observed to be much higher than normal. Whole blood copper levels appeared to correlate weakly with the estimated doses. Autopsy of the deceased patients showed ulceration of gastric and intestinal mucosa, liver cell necrosis and renal tubular cell necrosis.

An outbreak of gastroenteritis in 18/50 workmen following ingestion of tea made from copper sulfate-contaminated water was reported by Semple et al. (1960). Symptoms ranged from dizziness, headache, diarrhea, vomiting and abdominal pain to complete collapse. Water from a corroded geyser (heater) was later observed to contain $\geq$44 ppm total copper. Assuming each 70 kg man drank one cup (0.23 ℓ), the estimated dose was 0.143 mg Cu/kg. Nicholas and Brist (1968) reported a similar outbreak of toxicity from contaminated tea in factory workmen. Twenty workmen felt sick after ingesting morning tea made in a copper contaminated geyser. It was not stated how many men were exposed to the tea. Diarrhea occurred in 9/20, vomiting in 6/20 and nausea in 9/20. A sample of tea taken later contained 30 ppm Cu. It was stated that the copper concentration was probably higher. Assuming each man (70 kg) drank 1 cup (0.23 ℓ), the estimated dose was $\geq$0.1 mg/kg. There was no analysis for other metals or other contaminants.

TABLE VI-1

Human-Dose Effect Data for Copper

Sex/Age/ Number	Chemical	Route	Dose	Whole Blood Copper μg/100 mℓ	Serum Copper μg/100 mℓ	Effect	Comment	Reference
Male 32/1 Female 7,5/2	copper	oral	2-8 mg/ℓ Cu in drinking water	NR	NR	episodic emesis and abdominal pain in mornings	elevated copper in hair symptoms stopped when water source changed	Spitalny et al., 1984
Male and female/ 14-60/NR	copper sulfate	oral	1-5 g copper sulfate (0.25-1.3 g Cu^{+2})	383.4	264.6	metallic taste, nausea, vomiting and burning in epigastrium	normal whole blood copper ~217.0 μg/ 100 mℓ; normal serum copper = 151.6 μg/100 mℓ	Chuttani et al., 1965
Male and female/ 14-60/NR	copper sulfate	oral	6-15 g copper sulfate (1.5-3.8 g Cu^{+2})	519.0	332.4	metallic taste, nausea, vomiting and burning in epigastrium	normal whole blood copper ~217.0 μg/ 100 mℓ; normal serum copper = 151.6 μg/100 mℓ	Chuttani et al., 1965
Male and female/ 14-60/NR	copper sulfate	oral	16-30 g copper sulfate (4.1-7.6 g Cu^{+2})	684.0	346.2	metallic taste, nausea, vomiting and burning in epigastrium	normal whole blood copper ~217.0 μg/ 100 mℓ; normal serum copper = 151.6 μg/100 mℓ	Chuttani et al., 1965
Male and female/ 14-60/NR	copper sulfate	oral	>30 g copper sulfate (>7.6 g Cu^{+2})	605.5	239.5	metallic taste, nausea, vomiting and burning in epigastrium	normal whole blood copper ~217.0 μg/ 100 mℓ; normal serum copper = 151.6 μg/100 mℓ	Chuttani et al., 1965

TABLE VI-1 (cont.)

Sex/Age/ Number	Chemical	Route	Dose	Whole Blood Copper µg/100 mℓ	Serum Copper µg/100 mℓ	Effect	Comment	Reference
Male and female/ 14-60/48	copper sulfate	oral	1-30 g copper sulfate (0.25- >7.6 g Cu^{+2})	383.4- 605.5	264.6- 346.2	diarrhea in 14/48 jaundice in 11/48 hemoglobinuria or hematuria or both in 14/48 anuria in 13/48 oliguria in 5/48 hypotension in 4/48 coma in 4/48 death in 5/53	NC	Chuttani et al., 1965
Male/NR/20	copper sulfate	oral	30 ppm Cu^{+2} in tea ($\geq$6.9 mg Cu^{+2}) ($\geq$0.1 mg Cu/kg/day x 1 day)[a]	NR	NR	diarrhea in 9/20 nausea in 6/20 vomiting in 6/20	NC	Nicholas and Brist, 1968
NR/NR/150	copper sulfate	oral	44 ppm Cu^{+2} in tea ($\geq$10 mg Cu^{+2}) (~0.14 mg Cu/kg/day x 1 day)[a]	NR	NR	abdominal pain, vomiting, diarrhea, headache, dizziness in 10/150	NC	Semple et al., 1960
Female/NR/15	copper	oral	5.3-32 mg Cu (0.09-0.55 mg Cu/kg/day x 1 day)[b]	NR	NR	nausea, vomiting, diarrhea, abdominal cramps, headache in 10/15	patients ingested copper on empty stomach	Wyllie, 1957
Male/NR/3	copper metal	inhalation	$<$0.008 mg/m³	NR	NR	no metal fume fever	NC	Gleason, 1968
Male/NR/3	copper metal	inhalation	0.12 mg/m³ (0.84 mg/day or 0.012 mg/kg/day)	NR	NR	metal fume fever	NC	Gleason, 1968

[a] assume adult 70 kg men, one 8 oz cup (i.e., 0.23 ℓ) of tea

[b] assume adult 58 kg women

NR = Not reported

NC = No comment

Wyllie (1957) reported an outbreak of acute copper poisoning caused by the dissolution of copper contained in a cocktail shaker. Analysis of cocktail fluid prepared in the shaker allowed an estimate of the amounts of copper ingested (5.3-32 mg Cu). Fifteen adult females were exposed, and only five were asymptomatic. Weakness, abdominal cramps, headaches, nausea, dizziness and vomiting were experienced by the other 10 patients. Assuming these females weighed 58 kg, the estimated doses were 0.09-0.57 mg/kg/day. No measures or tests for other contaminants were reported. There are no values for serum copper or ceruloplasmin.

Walsh and Crosson (1977) reported a case of acute copper intoxication in an 18-month-old boy. After drinking a solution estimated to contain 3 g cupric sulfate (0.76 g Cu), the boy began to vomit and was admitted to a hospital. High copper levels were observed in serum (1.65 mg/100 mℓ) and urine (50 μg/100 mℓ) upon admittance. Acute hemolytic anemia developed on the day after admittance, at which time reduced glucose-6-phosphate dehydrogenase (G-6-DH) activity, hematuria, glycosuria and proteinuria were also noted.

Roberts (1956) reported that a well-nourished 24-year-old Negro male, after self-treatment with copper sulfate (~600 g over 4 months) was hospitalized with complaints of vomiting and gastrointestinal pain. The patient developed hemolytic anemia, but neither blood nor serum copper concentration was measured. The patient survived and was released after 2 weeks.

Acute copper poisoning in a 27-year-old man, resulting from the ingestion of at least 50 g of copper sulfate (12.7 g Cu) was reported by Chugh et al. (1975). In addition to emesis of his stomach contents, the patient was cyanotic, oliguric and anemic. Analysis of blood samples indicated a copper concentration of 8267 µg/100 mℓ, severe intravascular hemolysis and methemoglobinemia. A glucose-6-phosphate dehydrogenase assay of erythrocytes showed reduced activity of this enzyme. The patient suffered circulatory collapse and hypotensive shock and coma, and was reported to have died 16 hours after taking the chemical.

Sanghvi et al. (1957) reported two fatal cases of copper sulfate poisoning. Sulfhemoglobinenia and acute renal failure were observed in both patients, one of whom ingested about an ounce of copper sulfate and the other, an unknown amount. Both patients had sulfhemoglobin in their blood by 72 hours after ingestion of copper sulfate. Acute tubular necrosis and renal failure were observed in both patients, presumably caused by anoxia caused by sulfhemoglobinemia.

Stein et al. (1976) reported a case wherein a patient died after she was treated with an emetic dose of copper sulfate. A 44-year-old female ingested both alcohol and diazepam and was given 20 mℓ of a 10% solution of copper sulfate (508 mg Cu). The patient failed to vomit and stomach contents were removed by lavage. Respiratory collapse, hemolytic anemia, hemoglobinuria, hepatic failure, renal failure and massive gastrointestinal bleeding were observed, and the patient died. Rapid intestinal absorption of copper undoubtedly occurred, as she had a three-quarter gastrectomy. On autopsy, acute renal tubular necrosis and a liver copper level of 7.46 mg/100 g were observed.

Children may be particularly susceptible to copper toxicosis. Hepatic copper levels in normal newborns can be up to 8 times the levels in adults (Sternlieb, 1980). Two cases of copper poisoning in children have been reported. Salmon and Wright (1971) studied a nonfatal case of copper poisoning that initially appeared to be mercury poisoning. A 15-month-old boy was admitted to the hospital with prostration, vomiting, red extremities, hypotonia, photophobia and peripheral edema, but no mercury could be demonstrated. The outstanding abnormal finding was the elevated serum copper level (286 μg/100 mℓ); the plasma ceruloplasmin level was not measured directly but was estimated at 22.5 mg/100 mℓ. The copper content of the patient's drinking water was 0.35 and 0.8 mg Cu/ℓ in the cold and hot water taps, respectively. This water had been available to the patient for a period of 3 months, as this was the period of time that the family had occupied the residence. Apparently, the hot water system of the dwelling was entirely fabricated from copper, and the family used hot water for all cooking and beverages. The patient's ceruloplasmin level was normal, indicating that he was not suffering from Wilson's disease. The overall nutritional state of the patient and the presence of other contaminants in the water were not reported.

Walker-Smith and Blomfield (1973) reported a fatal case of a 14-month-old boy who may have been poisoned by excessive copper in drinking water both *in utero* and postpartum. Because Wilson's disease rarely manifests itself at such an early age, and there were no Kayser-Fleisher rings present (a diagnostic criterion for Wilson's disease), the authors were uncertain if this patient actually had Wilson's disease. However, the patient had a reduced ceruloplasmin level, raised urinary copper level and raised free plasma copper. These indicate the presence of Wilson's disease. The patient's household water supply had low pH and high copper levels. The cold water tap had copper concentrations of 6.8 and 0.3 mg/ℓ when first turned on and after running for 5 minutes, respectively. The hot water tap had copper concentrations of 9.7 and 6.9 mg/ℓ when first turned on and after running for 5 minutes, respectively. High copper concentrations were observed at autopsy in the liver, kidney, heart and brain of the patient. The authors suggested that the high environmental copper levels alone may have caused this death or the copper may have acted by exacerbating an existing liver infection or by overwhelming the patient's ability to remove copper because of Wilson's disease. The final diagnosis remained uncertain.

The Centers for Disease Control have reported 112 cases of copper intoxication from drinking water sources from the years 1977-1982. The majority of cases involved the leaching of copper from plumbing into drinking water with reported copper levels ranging from 4.0-70 mg/ℓ (CDC, 1977-1982).

Inhalation. There are several reports of local and systemic effects caused by the inhalation of copper sulfate (in Bordeaux mixture) by vineyard sprayers. Pimental and Marques (1969) were the first to recognize the disease they named "vineyard sprayer's lung." They reported their observations of two vineyard sprayers who had occupational exposure to Bordeaux mixture, a 1-2% solution of copper sulfate in hydrated lime used to control mildew on grapes. Two male vineyard sprayers were examined and found to have altered lung pathology similar to that seen in silicosis, although polarized light microscopy revealed the absence of silica. The lungs of these patients had a well-defined histological morphology including the presence of desquamation, intra-alveolar macrophages, inter-alveolar septal histiocytic granulomas and the scars of these lesions. Guinea pigs exposed to Bordeaux mixture by inhalation had similar lesions (see Chronic and Subchronic Toxicity in Chapter V).

Villar (1974) further defined the clinical aspects of vineyard sprayers' lung by analyzing 15 cases of the disease. Fourteen male patients and one female patient with a history of intermittent (3 months/year) exposure to Bordeaux mixture were admitted to a hospital for the diagnosis of a variety of pulmonary maladies, including cancer. Dyspnea, weakness, appetite loss, weight loss, radiographic opacities and the presence of copper in the lungs were observed to be common among these patients. In some cases that were followed for 3 years, retraction and calcification of lesions previously described as tumor-like opacities were observed. The authors were able to classify the various manifestations of the disease into three separate groups: subclinical, active and chronic.

Pimental and Menezes (1975) reported an additional adverse effect of occupational exposure to aerosols of Bordeaux mixture which contains aresenic compounds as well as copper. Copper containing liver granulomas were observed in three cases of vineyard sprayers' lung, along with the characteristic pulmonary lesions of the disease. Exposure to Bordeaux mixture ranged from 3-15 years duration. Liver specimens were collected at autopsy in two cases and by percutaneous biopsy in the third. Well-defined nodules composed of histiocytic cells, granulomas organized in sarcoid-type follicles and neoformation of reticulin fibers were observed in the livers of these patients. Tests for copper showed that the metal was present in granulomas and fibro-hyaline nodular scars, and this finding differentiates the observed lesions with those observed in other conditions.

Three workmen involved in polishing copper plates were exposed to fine copper dust and complained of stuffiness of the head, sensations of chills or warmth and a general feeling of discomfort, similar to the onset of a cold (Gleason, 1968). These symptoms, when present after exposure to metal fumes, are generally known as metal fume fever. While the copper dust in question was not fume per se (i.e., airborne particles condensed from the molten state), they were observed to be of "extreme fineness." General air samples in the workplace contained 0.030-0.075 mg Cu/m^3. A breathing zone air sample of the polishing wheel operator contained 0.120 mg Cu/m^3. These concentrations were associated with metal fume fever. After the installation of engineering controls (water spray and local exhaust ventilation), copper concentrations were reduced to <0.008 mg/m^3 and no further symptoms of metal fume fever were observed.

Earlier reports of acute poisoning from inhalation of copper-containing dust are summarized in Browning (1961). Dusts of metallic oxides and Cu were reported to have caused symptoms of heavy metal poisoning, pain in the chest, dyspnea, nausea and vomiting.

Dermal Absorption. Holtzman et al. (1966) reported the death of a burn patient who absorbed large amounts of copper through her skin during debridement with copper sulfate crystals. The patient (5 years 6 months old) had suffered second and third degree burns over 30-40% of her body. She underwent seven separate debridement procedures ~1 week apart. Following the debridement procedures, the patient was pale, anemic, icteric, oliguric and jaundiced. Copper levels in the patient's urine and serum were

220 and 540 μg/100 mℓ, respectively. Erythrocyte glucose-6-phosphate dehydrogenase was not reduced and serum ceruloplasmin was elevated to 86 mg/100 mℓ.

Epidemiological Studies

Several epidemiological studies of workers involved in copper smelting have reported that the incidence of lung cancer is increased in this population. However, it is believed that the etiologic agents do not include copper; rather, arsenicals such as arsenic trioxide may be responsible.

Some areas of North Wales, Cheshire and Devonshire have a high prevalence of gastric carcinoma. It was observed that these areas also have increased ratios of zinc to copper in the soil (Stocks and Davies, 1964). Copper intake (also zinc, cadmium and chromium) was observed to be correlated with the incidence of leukemia and other cancers (Schrauzer et al., 1977). These studies, as all such geographical correlation studies, are designed to generate testable hypotheses. They are inconclusive regarding the role of copper in human carcinogenicity because of the grouped exposure and response data.

High Risk Subpopulations

Wilson's Disease Patients. Hepatolenticular degeneration (also known as Wilson's disease) was described as a clinical syndrome by Wilson in 1912 (Williams, 1982). Wilson's disease is probably the best studied form of copper toxicity in man, and this effort has provided much information regarding human copper metabolism (Scheinberg and Sternlieb, 1969). This disease is an inherited autosomal recessive disorder of copper metabolism characterized by abnormally low levels of ceruloplasmin, increased plasma copper levels, hypercupuria and increased copper deposition in liver, brain, kidneys and cornea (Schroeder et al., 1966; Evans, 1973). A rare disorder, Wilson's disease occurs in perhaps 1 of 200,000 individuals (Scheinberg and Sternlieb, 1969). The prevalence of persons heterozygous for Wilson's disease may be ~1 in 200 (Sternlieb, 1980). Wilson's disease patients may be particularly susceptible to the toxic effects of copper from environmental sources.

In Wilson's disease, the liver may concentrate up to 20 times the normal level of copper. This eventually destroys parenchymal cells, and their copper content is thus released into the blood. Once released, copper may affect many organs and systems, including erythrocytes, kidneys, cornea and the CNS (Scheinberg and Sternlieb, 1969).

Untreated Wilson's disease patients may suffer from tremors, drooling, incoordination, seizures, behavioral abnormalities, anemia, jaundice and, ultimately, death. Chelating agents, such as d-penicillimine and British Anti Lewisite (BAL) have been used to control the disease. However, the efficiency of penicillimine for increasing urine copper is low. Limitation of copper intake and administration of potassium sulfide are also used to reduce copper absorption (Schroeder et al., 1966).

Wilson's patients should measure the copper level in their drinking water because soft, acidic or basic water may dissolve copper from copper tubing in residential plumbing.

Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency. Excessive copper has been demonstrated to reduce the activity of the hexose monophosphate shunt, of which G6PD apparently plays a role. This has been demonstrated in Wilson's disease patients (Diess et al., 1970), in *in vitro* incubations with human erythrocytes (Boulard et al., 1975; Calabrese et al., 1980) and in copper sulfate acute poisoning cases in humans (Walsh et al., 1977; Chugh et al., 1975). The concentrations of Cu in the *in vitro* experiments are much higher than those encountered *in vivo*.

It is reported that 13% of the American Negro male population have G6PD deficiency and may be at increased risk to the toxic effects of copper (Calabrese et al., 1980; Calabrese and Moore, 1979). Boulard et al. (1975) observed that the G6PD activity of human erythrocytes is inhibited when incubated with 0.1 mM copper.

Kidney Dialysis Patients. Several reports of acute hemolytic anemia in patients undergoing hemodialysis have been attributed to excess copper in the dialysis fluid (Williams, 1982). Leaching of copper from copper tubing in home units and from copper filters and tubing within units have both been reported. Increased copper concentrations are associated with soft water and acidic water. This effect has been observed at copper concentrations from 22-50 μg/ℓ in the dialysate (Owen, 1981). The problem can be controlled by removing copper from the system and monitoring the pH and conductivity of the dialysate (Williams, 1982).

Occupational Exposure. Inhaled copper aerosols have been demonstrated to cause local lesions, metal fume fever and hepatic accumulation of the metal (Pimental and Menezes, 1975). Persons involved in spraying of copper sulfate mixtures in vineyards or with occupational exposure to copper dusts and fumes may be at additional risk for the development of alveolar cell carcinoma and cirrhosis of the liver (Pimental and Menezes, 1977). It is not known what effect elevated oral intake of copper may have in this population.

Summary

The symptomatology and effects of acute copper poisoning have been characterized (Chuttani et al., 1965). Effects such as a metallic taste, vomiting, diarrhea and epigastric pain have been reported at acute exposes to copper in concentrations as low as 5.3-32 mg of copper (Semple et al., 1960; Wyllie, 1957; Spitalny et al., 1984). Of this only Spitalney et al. describes exposure that was not to copper sulfate. Burch et al. (1975) observed that a single dose of 15 mg Cu usually induces symptoms. Copper sulfate historically was used as an emetic at a dose of 300 mg (76.2 mg Cu), but this use has been discontinued. Copper sulfate is included in many vitamin supplement tablets (0.5-2 mg Cu) and has been used as a hematinic at adult doses of 15-30 mg/day (3.8-7.6 mg Cu/day) (Hoover, 1970).

Severe effects of acute copper intoxication have been reported, including hemolytic anemia, G6PD inhibition, renal failure, hematuria and death (Walsh et al., 1977; Roberts, 1956; Chugh et al., 1975). These effects were reported to occur at doses between 3 and 600 g of copper sulfate (0.76-152.4 g Cu).

Chronic copper toxicity is rarely encountered in humans. Long-term exposure to aerosols of Bordeaux mixture ($CuSO_4$ and lime) has been reported to cause adverse effects on pulmonary and liver morphology and may be associated with tumor formation (Pimental and Marques, 1969; Pimental and Menezes, 1975; Villar, 1974). Inhalation of copper fumes and fine aerosols may result in metal fume fever (Gleason, 1968).

Epidemiological investigations of populations with high copper exposure by inhalation are equivocal with respect to the carcinogenicity of the metal, because the exposures encountered by copper miners and smelters usually involve other metals including arsenic known to contribute to lung cancer.

Wilson's disease (hepatolenticular degeneration), a rare inborn error of copper metabolism, appears to be the only manifested form of chronic copper toxicity by ingestion in humans. Elevated environmental copper levels may enhance the toxic effects of the metal but no quantitative data are available. Left untreated, the disease can be fatal. Copper is accumulated in the liver, brain, kidney and cornea of Wilson's disease patients (Evans, 1973). Cirrhosis of the liver, hemolytic anemia, neurological abnormalities and corneal opacities (Kayser-Fleischer rings) are some of the marked adverse effects of copper observed in Wilson's disease.

It has been suggested that persons with Wilson's disease, deficiencies in G6PD activity, and those having occupational exposure to copper may be at additional risk from the toxic effects of copper (U.S. EPA, 1980b).

It is entirely possible to prepare an adequate diet containing >10 mg copper that has satisfactory caloric value (Schroeder et al., 1966). This undoubtedly occurs frequently in normal individuals without adverse effects.

VII. MECHANISMS OF TOXICITY

The metabolism and toxicity of copper and other heavy metals are controlled by many complex biochemical reactions. In addition to copper's affinity to become bound to proteins, the metal in both its cupric (+1) or cuprous (+2) form is essential for the proper functioning of many enzyme systems. Copper levels in various human tissues are affected by age, sex, nutritional status, disease state and a wide variety of other factors.

Transition metals (such as copper) are known to catalyze lipid peroxidation, possibly forming free radicals (Dougherty and Hoekstra, 1982). However, copper is usually incorporated into stable complexes within cells or vascular fluids (Evans, 1973).

The wide ranging effects of copper in normal metabolism have been known for some time. Fluctuations in serum copper levels in pregnancy were first observed in 1928; and since that time, changes in the various copper proteins and enzymes with various biological states have been documented. Copper homeostasis is affected by a variety of hormones. Several pituitary, adrenal, thyroid and sex gland hormones have been observed to cause effects in copper metabolism (Evans, 1973).

The daily balance of copper in normal humans is dependent upon a variety of variables, including age, sex and diet. Some metals, cadmium, zinc, silver and molybdenum, may influence the metabolism of copper by altering its absorption or cellular utilization (Evans, 1973).

Hepatotoxicity

When homeostatic mechanisms are overwhelmed by excess copper exposure, accumulation of the metal in the liver results. Chronic copper toxicity in sheep, rats and man is associated with an absence of outward symptoms during a phase of constant accumulation of hepatic copper (Helman et al., 1983). Lal and Sourkes (1971) observed that during the asymptomatic phase of copper toxicity in rats, copper accumulated in all subcellular hepatic fractions.

Animal Model for Hepatotoxicity. The beige and conventional strains of $C57B_1/6$ mice were used to study the effects of copper administration on the intracellular compartmentalization of copper and hepatotoxicity. Beige mice possess hepatocytes with altered phagocytic capacity to scavenge metal proteins and sequester them in lysosomes. Both strains received i.p. injections of 8 mg Cu/kg/day for up to 4 weeks. After exposure, livers were removed, homogenized and fractionated by centrifugation. Analysis of the various fractions showed that copper initially accumulated in the cytosol fraction of both strains, but only the conventional mice had subsequent binding of copper to the lysosomal fractions. On histological examination, beige mice were observed to have more severe hepatic lesions than conventional mice. It was concluded that sequestration of copper into lysosomes by thiol rich macromolecules (metallothioeins) is a normal protective mechanism. In beige mice, copper accumulated in cytosol, saturated the available binding sites, was not sequestered into lysosomes and caused cellular injury (Helman et al., 1983).

Blood Toxicity

Cellular membranes can be adversely affected by copper. The metal has the ability to catalyze lipid peroxidation, to inhibit membrane ATPase

(Evans, 1973) and to increase the osmotic fragility of erythrocytes (Moroff et al., 1974). The exact mechanism of copper's effect on cell membranes is not known, but it is believed to function directly, by binding to exterior sites, or indirectly by causing intracellular changes that affect membrane integrity.

Erythrocytes are susceptible to the toxic effects of copper. It is known that copper can produce homolysis, reduce blood glutathione, increase methemoglobin, induce formation of Heinz bodies, reduce G6PD activity and possibly catalyze the autooxidation of glutathione (Chuttani et al., 1965; Bremner, 1979).

Glucose 6-Phosphate Dehydrogenase Deficiency (G6PD). Calabrese and Moore (1979) postulated a mechanism describing copper-induced hemolysis. Copper can inhibit both glutathione reductase and G6PD. Decreased levels of reduced glutathione in erythrocytes have been associated with their susceptibility to hemolysis. Boulard et al. (1975) demonstrated that a variety of erythrocyte enzymes are inhibited when incubated with low levels of copper (0.1 mM). It is not known whether the "so called acquired enzymatic defects" found in various chronic disorders are a cause or effect of the simultaneous high levels of serum copper.

Animal Models -- Dorset strain sheep (Calabrese et al., 1980) have erythrocytes with low G6PD activity and sensitivity to primaquine. These traits are possessed by human erythrocytes deficient in G6PD. Increased methemoglobin, decreased GSH and decreased acetylcholinesterase (ACH) activity have been observed in both types of cells when incubated with 2 mM copper.

Wilson's Disease

Medical study of the nature of Wilson's disease has afforded a unique understanding of the pathogenesis of chronic copper intoxication.

Wilson's disease is characterized by decreased plasma copper and ceruloplasmin, increased amounts of amino acid and albumin bound copper, increased urinary excretion of copper, and deposition of copper in the liver, brain and cornea. The effects of chronic copper poisoning in Wilson's disease patients include hepatic cirrhosis, kidney defects, hemolysis, brain damage and nerve demyelination (Stokinger, 1980). Patients with this disease represent probably the only form of chronic copper toxicosis in humans. The available evidence suggests that copper deposition in tissues is related to the developmental mechanism of the disease (Narasaki, 1980).

While the exact mechanisms of Wilson's disease have not been elucidated, these studies illustrate the complexity of the copper homeostatic mechanisms in normal man.

Animal Models. Bedlington terriers are similar to Wilson's disease patients because the abnormal copper metabolism in both is transmitted by an autosomal recessive inheritance (Sternlieb, 1980). Accumulation of tissue copper leading to hepatitis, cirrhosis and anemia is common to both. Bedlington terriers are a good model to study liver toxicosis, but do not emulate Wilson's disease patients in other respects. For example, the dogs do not have decreased ceruloplasmin levels, and do not develop Kayser-Fleisher rings (Sternlieb, 1980).

Cattle and sheep with chronic copper poisoning (especially those with enzootic jaundice) undergo hemolysis in a manner quite similar to patients with Wilson's disease. For example, hemolysis occurs after large tissue stores and high urine copper are observed. The quick release of copper from the liver at this time may induce the hemolytic effect (Diess et al., 1970).

Menkes Syndrome

Menkes syndrome is an X-linked inherited defect in copper absorption, leading to severe deficiency states (Danks et al., 1972). Insufficient intestinal absorption of copper was proposed by these authors as the basic biochemical defect of the disease.

Animal Model. The mottled mutant mouse provides an excellent animal model for studying Menkes disease. Hemizygous males with the X-linked allele, brindle (Mo^{br}/y), possess some of the severe defects observed in Menkes disease. These include hypopigmentation, skeletal and vascular defects, abnormal hair, and early death (Williams, 1982).

Summary

The human homeostatic mechanisms controlling copper balance can store copper, prevent its excess absorption from the gut, utilize the metal in cellular function, and excrete the metal through the bile or urine. Copper can bind to proteins and is essential for many enzyme functions. The metabolism of copper as well as serum copper levels vary by age, sex and diet. Homeostatic control prevents toxicity from the wide normal variations in copper. In the cases of excess, copper accumulates in the liver, producing no outward symptoms of toxicity. When copper levels increase in the blood, toxic effects on erythrocytes and cell metabolism are seen. A form of chronic toxicosis (Wilson's disease) is a rare genetic error of metabolism that leads to accumulation of copper in tissues, hemolysis cirrhosis, neurological abnormalities and renal damage.

Copper is rarely toxic when ingested, unless very high amounts are absorbed. Acute ingestion of copper compounds can cause gastrointestinal disturbances, hemolysis, cirrhosis, renal damage and death.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an acceptable daily intake (ADI) is calculated. An adjusted acceptable daily intake (AADI) and health advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{\text{(NOAEL or LOAEL in mg/kg/day) (body weight in kg)}}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking water volume in } \ell\text{/day}} = \text{mg/}\ell$$

where: NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
Body weight = 70 kg for adult or 10 kg for child
Drinking water volume = 2 ℓ/day for adults or 1 ℓ/day for children
Uncertainty/Safety Factor = 10, 100 or 1000

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A 1-day HA for 10 kg child
2. A 1-day HA for 70 kg adult
3. A 10-day HA for 10 kg child
4. A 10-day HA for 70 kg adult
5. A lifetime AADI for a 70 kg adult

The distinctions made between the HA calculations (items 1-4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1-2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivities to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS, 1977, 1980) are typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.
- An uncertainty factor of 1000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data identify a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines (NRC, 1977) for selection of an uncertainty factor, but must be considered, include the following: 1) the quality of the toxicology data, 2) the significance of the adverse effect, and 3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen (copper is not a carcinogen), mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical by drinking water. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for noncontinuous animal feeding, nonlifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 ℓ of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as 1) incomplete knowledge concerning the health effects of contaminants in drinking water, 2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results, and 3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects

Short-term oral administration of copper to rats results in accumulation of the metal in hepatic and renal tissue and necrosis of these tissues (Boyden et al., 1938; Haywood, 1980; Rana and Kumar, 1980). Boyden et al. (1938) studied the effects of feeding high levels of copper to albino rats for 4 weeks. Groups of 3-5 mixed male and female 28-day-old albino rats were maintained on diets supplemented with 0, 500, 1000, 2000 or 4000 ppm copper (as copper sulfate) for 4 weeks. The authors stated that these dosage levels resulted in average copper intakes of 0, 5.1, 8.2, 10.8 and 7.6 mg/rat/day based on observed food consumption. Animal body weight was recorded weekly. At the end of the 4th week of exposure, blood samples were taken, the rats were killed and their gross pathology was examined. The livers and spleens were removed for analysis of copper content. All rats maintained on the 4000 ppm diet died within 1 week, but had average food intake when compared with other groups. Reduced body weight gain and food intake and jaundice were observed in rats maintained on diets containing 2000 ppm copper. Reduced body weight gain and food intake were observed in rats maintained on diets containing 1000 or 500 ppm copper. Greater copper accumulation in the blood, spleen and especially liver in comparison with the control group was observed in all treated rats. The authors stated that slight toxicity was observed in rats fed 500 ppm and increased toxicity was observed as the dietary copper level was increased (see Table V-1).

Haywood (1980) maintained rats (4 male weanling rats/group) for 1, 2, 3, 6, 9 or 15 weeks on diets containing copper at levels of 2000 mg/kg (as copper sulfate). Histologic descriptions of the liver and kidneys were provided for each week of exposure. At the end of week 1, there was no copper in the liver, and no histopathologic changes were seen; at week 2, there was copper in the outer zone of the liver, and periportal hypertrophy of the parenchymal cells was observed; at week 3, there was marked copper deposition, and inflammatory foci were seen. The lesions were most severe at week 6, when severe necrosis was observed. By week 9, regeneration that limited the necrotic area was observed, and copper deposition was less pronounced. At the last sacrifice period, liver regeneration was still evident and diffuse fibrosis was observed. Changes in the histopathology and copper content of the kidneys followed the same pattern as described for the liver, with almost complete recovery observed after 15 weeks of exposure.

Rana and Kumar (1980) administered lower exposure levels of 100 mg/kg bw copper sulfate by gavage to 20 male rats (90 days old). During the 21 days of treatment, the copper-fed rats had decreased skeletal development and decreased body, liver and kidney weights; however, no histopathologic changes were observed in the liver or kidneys. In studies using slightly lower exposures, Miranda et al. (1981) observed no changes in serum enzymes indicative of liver damage in rats (number not specified) exposed to copper at a level of 55 mg/kg diet for 5 weeks. The only effect of treatment was an increase in liver copper content.

The Centers for Disease Control have reported 112 cases of copper intoxication from drinking water sources from the years 1977-1982. The majority of cases involved the leaching of copper from plumbing into drinking water with reported copper levels ranging from 4.0-70 mg/ℓ (CDC, 1977-1982). Thus, exposures of humans to Cu in the drinking water is associated with disease. While this is important in view of the numerous reports of effects of copper sulfate, these solutions may in some cases have contained other contaminants.

In a study concerned with the effects of copper on reproduction, Lecyk (1980) fed two groups of 7-22 female mice (either C57BL or DBA mice) diets containing copper sulfate at levels of 0, 0.5, 1.0, 1.5, 2.0, 3.0 or 4.0 g/kg. After 1 month, these females were mated with males also exposed to copper sulfate, and at day 19 of gestation, the females were killed. The two lower doses resulted in stimulation of embryonic development (fetal weight and litter size); however, at 1.5 g/kg fetal development was decreased as compared with the highest index observed, and at 2000 mg/kg measures of fetal development were decreased as compared with control values. Although low levels of copper sulfate (0.5-1.0 g/kg diet) appeared to be beneficial to fetal development, higher levels produced adverse effects.

In pigs (groups of 8-12) given diets containing copper at levels of 150, 200 or 250 ppm for 61-88 days, there was accelerated weight gain (Kline et al., 1971). At the next higher dose level of 500 ppm, weight gain was decreased compared with controls. Also at the higher dose level, hemoglobin and hematocrit levels were reduced. In a second study in pigs (Suttle and Mills, 1966a,b), however, exposure of female weanling pigs (6/group) to diets containing 250 ppm of copper for 79 days resulted in increased aspartate transaminase activity and jaundice. Higher levels of 600 ppm for 48 days resulted in anemia. The results in the lower exposure group may have been partially caused by high levels of calcium in the diet.

There is only one chronic animal study of copper. In this study, Howell (1959) maintained 5 male and 5 female rats on diets containing 5000 ppm of copper acetate for 16 months. At necropsy, the distribution of copper was reported to be primarily to the liver and kidney. It appears that toxicologic endpoints were not evaluated in this study.

Chuttani et al. (1965) evaluated case reports of acute copper sulfate poisoning survivors (48 cases) and deaths (5 cases). They observed that 48/48 patients ingesting from 250 mg to >7.6 g of Cu had common symptoms: metallic taste, nausea, vomiting and gastrointestinal disturbance. Diarrhea, nausea and vomiting were experienced in human populations of 150, 20 and 15 persons who ingested between 5.3 and $\geq$10 mg Cu. Semple et al. (1960) reported that 18/150 factory workers ingesting tea containing at least 44 ppm (44 mg Cu/ℓ) experienced nausea, vomiting and diarrhea. Assuming these were 70 kg adult male workers and that they ingested ~8 ounces of tea (0.23 ℓ), the approximate dose was 10 mg for one exposure. Using the same assumptions, Nicholas and Brist (1968) reported a similar outbreak in tea drunk by at least 20 workmen. Tea water was observed to contain 25-30 ppm (25-30 mg Cu/ℓ) and the estimated single dose, $\geq$7.0 mg, caused diarrhea in 9/20, nausea in 9/20 and vomiting in 6/20. Wyllie (1957) reported that 10/15 "fasted" adult females ingested alcoholic drinks resulting in between 5.3 and 32 mg Cu/person had nausea, vomiting and diarrhea.

Spitalny et al. (1984) reported that three members of a family suffered acute copper intoxication characterized by nausea and stomach cramps, after ingesting water with copper levels of 2.8-7.8 mg/ℓ. This occurred on several mornings after ingestion of an unspecified amount of drinking water and juice. The water consumed was quite corrosive having a Langelier Index of -3.92 and an Aggressive Index of 8.93.

Quantification of Noncarcinogenic Effects

Calculation of the 1-Day HA. At high dietary levels of 2000 mg Cu/kg, Haywood (1980) observed the beginning of copper accumulation in the liver and kidneys of rats by 14 days. Jaundice was observed in rats fed diets containing 2000 or 4000 ppm copper. Lower exposures of 500 or 1000 ppm reduced body weight gain and food intake (Boyden et al., 1938).

The available human studies of Chuttani et al. (1965), Semple et al. (1960) and Wyllie (1957) all suggest that ingestion of between 5.3 and 32 mg copper/person results in gastrointestinal disorders, vomiting, nausea and diarrhea. Since no lasting adverse effects were reported and the symptoms observed could be the result of local gastrointestinal irritation, the single oral dose of 5.3 mg may be considered a LOAEL. The human data describe lower exposure levels than the animal data and do not require species to species extrapolation. Thus, these human data would be the most appropriate for deriving the 1-day HA.

The calculation of the 1-day HA for an adult from this human LOAEL is as follows:

$$\begin{aligned} \text{1-day HA} &= 5.3\ \text{mg/day} \div 2\ \ell\text{/day} \div 2 \\ &= 1.3\ \text{mg}/\ell \end{aligned}$$

where

5.3 mg = the total dose ingested at one time

2 ℓ = amount of water consumed in ℓ/day by an adult.

2 = uncertainty factor

According to the NAS guidelines, an uncertainty factor of 10 should be applied when using calculations based upon a human study with a NOAEL. In this situation, an uncertainty factor of 2 was applied because of the following: 1) the symptom reported was local gastrointestinal irritation and was not permanent; no long-term effects were reported; 2) 5.3 mg/day was the lowest value determined in the literature based upon a number of studies. The value was based upon fasted subjects and thus is a very conservative number; 3) copper is an essential element and the use of a larger safety factor would bring the level below that considered necessary for human nutrition; and 4) copper absorption is controlled by a homeostatic mechanism and the chemical does not tend to accumulate in the body. Further support for this number as conservative is obtained from the incident reported by Spitalny et al. (1984) in which acute toxicity occurred after water was ingested that had >2 mg/ℓ and in which exposure may have been occurring over a period of several months.

The 1-day HA for a 10 kg child based on this human LOAEL should be the same as that for the adult since the progression of symptoms, vomiting occurring soon after exposure, suggests that systemic absorption had not occurred. If effects observed are considered local in origin, then it would not be appropriate to distribute the dose over the body weight. The 1-day HA for the child is also 1.3 mg/ℓ.

Calculation of 10-Day HA. As described previously for the 1-day HA, Haywood (1980) observed transient liver damage, which was at maximal severity at 6 weeks, in rats exposed for 15 weeks to copper at doses of 100 mg/kg bw. In studies approaching 90 days in pigs, dietary exposure to copper at doses of ~3.2 mg Cu/kg bw/day was reported to accelerate body weight gain (Kline et al., 1971), while a similar dose in pigs of 2.6 mg Cu/kg bw/day was reported to result in jaundice and increases in serum aspartate transaminase levels (Suttle and Mills, 1966a,b). The results of the second study were possibly confounded by high calcium levels in the diet. Interactions between metals and other natural dietary components are not uncommon.

A 10-day HA will not be calculated for copper because of inadequate data. The available animal data do not consist of dose-response information that adequately defines a NOAEL or a LOAEL. The Haywood (1980) study maintained exposure at one high dose and did not explore lower doses, while the other available animal studies also consisted of one dose exposures. The human data consists solely of one-time exposure information.

Calculation of Lifetime AADI. Howell (1959) observed that rats maintained on diets containing 5000 mg Cu acetate/kg/day for up to 16 months had copper deposition in renal and hepatic tissue. However, no other toxicological effects were investigated, and it is not possible to state whether 5000 mg Cu acetate/kg/day represents a LOAEL. Additional data for chronic oral toxicity in nonruminant laboratory mammals were not found in the available literature.

There are no data indicating that human exposure to copper results in chronic toxic effects. The available information on human copper toxicity consists of data on gastrointestinal effects resulting from acute copper toxicity. These data were used to calculate the 1-day HA. Additional data for chronic oral toxicity in non-ruminant laboratory mammals were not found in the available literature, and there are no data for chronic human exposure to copper. Thus, the 1-day HA of 1.3 mg/ℓ will also be used as the AADI, since acute effects appear to be the effects of concern from exposure to copper.

It should be noted that the 1-day HA and the AADI used a LOAEL of 5.3 mg, based upon the lowest single oral dose of copper resulting in adverse effects as reported in the literature. A number of studies have shown the ingestion of copper at levels in this general range to result in adverse effects. Sargent and Jean (1983) reported acute copper intoxication after ingestion of water with copper levels of 5.6-7.8 mg/ℓ. This occurred in the morning hours after ingestion of drinking water and juice. The exact amount of water consumed was not reported; however, the age of two of the affected family members (5 and 7) makes it improbable that a large quantity was consumed. It appears likely that <1 ℓ was ingested and thus it may be assumed that a dose <5.6 mg resulted in acute copper toxicity. The CDC have also reported a number of cases of acute copper intoxication from drinking water sources. The exact level of copper in the water was not reported for many of the incidents, however, levels ranging from 4.0-70 mg/ℓ were reported for a number of cases (CDC, 1977-1982).

The average adult copper intake through the diet is variable, although it appears to be <2 mg/day (NAS, 1980b). Absorption of inhaled copper probably contributes little to the overall daily intake of copper (U.S. EPA, 1980b). Schroeder (1970) estimated that inhalation of ambient air would contribute <1% of the total normal daily intake (Schroeder, 1970). Other sources (copper bracelets, IUDs) are also expected to contribute little to total intake (U.S. EPA, 1980b).

In addition, the taste and odor of copper in drinking water serves to limit excess intake. Cohen et al. (1960) determined the copper concentrations added to distilled water required to elicit taste sensations in a population (15-20) of human volunteers. The concentrations of Cu^{2} ion in distilled water necessary to cause a threshold taste sensation in 5% and 95% of a panel were 2.6 and 15.8 ppm (2.6 and 15.8 mg/ℓ), respectively. Copper was generally described as having a bitter taste (Cohen et al., 1960). In setting standards for copper levels in ambient water, the Federal Water Quality Administration (FWQA, 1968) based their acceptable limit on these organoleptic properties of copper dissolved in water. This limit was 1 mg/ℓ. The U.S. EPA (1980b) also recommended an ambient water quality criterion for copper of 1 mg/ℓ based on organoleptic considerations and the U.S. EPA (1979) also set a secondary drinking water standard of 1 mg/ℓ based on the taste and odor considerations. The World Health Organization (WHO, 1984) also recommends 1.0 mg/ℓ as a guideline for drinking water quality.

A level of 1.3 mg/ℓ is recommended as the ADDI based upon the following: 1) this level would satisfy the nutritional requirements for copper; the National Academy of Sciences (NAS, 1980b) estimated that "an adequate and safe" intake of 2-3 mg copper in a 70 kg adult will satisfy nutritional requirements and be protective of human health; and 2) assuming consumption of 2 ℓ of water per day, 1.3 mg/ℓ copper in the drinking water would result in a daily intake of less than the lowest levels that were seen to result in gastrointestinal effects in humans (5.3 mg/day, 3-8 mg/ℓ). This value would thus be protective against acute toxic effects in humans. This value is not protective against copper toxicity in sensitive members of the population, such as those rare individuals with Wilson's Disease. These individuals would have to further limit their intake of copper from all sources.

Carcinogenic Effects

A special complex of copper, copper 8-hydroxyquinoline, when administered by a single subcutaneous injection gave significantly increased incidence of reticulum cell sarcomas only in male $B6C3F_1$ mice.

Mutagenicity studies have reported mixed results. No evaluation of the carcinogenicity of copper or copper compounds can be made on the basis of the available data.

Quantification of Carcinogenic Effect

Excess carcinogenic risk values have not been calculated for copper.

Special Considerations

Copper tubing is widely used in home plumbing systems. The variables that may affect copper levels in drinking water are degrees of water pH, softeners, temperature, duration of contact with copper tubing and grounding of electrical systems to copper pipes.

Synergistic Effects. The mechanistic nature of the metabolic interactions between copper and molybdenum, calcium, sulfate, iron and zinc in animal and man is not thoroughly understood. Copper toxicity in swine may be reduced with the concurrent administration of zinc and iron, and copper has a prophylactic effect on rats concurrently exposed to toxic levels of zinc (NAS, 1977).

Special Groups At Risk. Untreated Wilson's Disease will result in chronic toxicity even at normal levels of copper intake (2-3 mg/day). Walker-Smith and Blomfield (1973) reported a case wherein a child with suspected undiagnosed Wilson's Disease died from exposure to 6.9-9.7 mg/ℓ of drinking water for 14 months.

Kidney dialysis patients may be susceptible to acute toxicity from dialysis fluid containing excess copper. Such exposure may have an adverse effect on erythrocyte membranes and enzyme systems.

Persons with G6PD deficiency are likely to be susceptible to toxic effects of oxidative stressors (Calabrese and Moore, 1979) such as copper. Inasmuch as ~13% of the American Black male population may have this deficiency, along with other populations, elevated copper in their drinking water may place these persons at special risk.

Summary

Copper is recognized as an essential element. About 2-3 mg/day are required for proper nutrition. Human exposure to copper has been demonstrated to cause acute toxicity. Chronic effects are not likely because of the acute toxicity limits intake and because copper is an essential element controlled by a homeostatic mechanism and does not tend to bioaccumulate.

The lowest level of copper exposure necessary to cause this toxicity was a single oral dose of 5.3 mg. A 1-day HA of 1.3 mg/ℓ is associated with this dose and is appropriate for both children and adults.

The data base for copper was insufficient to derive a 10-day HA. An AADI of 1.3 mg/ℓ was also determined based upon acute toxicity effects in humans.

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